THE

JOURNAL OF

COMPARATIVE PATHOLOGY

AND

THERAPEUTICS.

Vol. XLIII.—No. 1. MARCH 31, 1929. PRICE 3s. 6d.

B. Paludis: A NEW SPECIES OF PATHOGENIC ANÆROBIC BACTERIUM.

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THE bacterium which is described in this paper has been encountered on several occasions during investigations on diseases of sheep on the Romney Marsh, Kent. The particular diseases are known locally by the terms "struck" and "gangrene." The term "struck" is applied by the farming community to a rapid and fatal disease where post-mortem examination reveals an acute inflammatory condition in one or more of the following parts of the body: areas of muscular tissue, organs in the abdominal cavity, and organs in the thoracic cavity. The term "gangrene" applies to a similar disease in ewes attacked within a few days after lambing.

From the investigations carried out during the past two years, evidence shows that a number of species of pathogenic sporulating anærobic bacteria may each separately be responsible for these diseases.

The particular bacterium now under consideration is one of the number of species encountered, and is described separately because it is of peculiar interest, as it has been recovered from the muscular tissue where the lesions were in many ways similar to those of black-quarter, and because the bacteria resemble *B. welchii*, and may readily be mistaken for that micro-organism unless careful and detailed bacteriological examinations are made.

The bacteria probably belong to a hitherto unrecognised species, and if this be so, I suggest that the bacterium should be named *B. paludis*; this name is proposed because the bacteria have been found causing disease in animals grazing and living on a marsh.

Material From which *B. paludis* was Isolated.

In each instance the material consisted of muscular tissue which was forwarded to the laboratory in sterile containers.

Given below, for each case, are the descriptions of the lesions in the sheep as tabulated by the owner ; the appearance of the specimen when received at the laboratory, together with the particulars of microscopical examinations of smears made from the muscle ; the preliminary cultural findings ; and the results of guinea-pig inoculations.

Specimen No. 14.

This consisted of a portion of muscle, dull red in colour, soft, and friable. Particulars of the post-mortem examination were not received. Smears showed numerous moderately large, stout, stumpy bacilli with bluntly rounded ends ; no spores were seen ; the bacilli were Gram-positive.

Cultures showed that ærobic contaminating bacteria were present, and pure cultures of *B. paludis* were not obtained by direct cultural means. A guinea-pig inoculated subcutaneously with 0·1 c.c. of a mixed broth culture died, and from its heart blood *B. paludis* was isolated.

Specimen No. 29.

The owner reported that the subcutaneous tissue, the muscular tissue, and the stomach and intestines were affected. When the muscle was received it was deep red in colour, and obviously diseased. Smears showed an apparently pure and rich culture of non-sporulating bacilli similar to those seen in smears from Specimen No. 14. *B. paludis* was recovered in pure culture from the muscle ; no other bacteria were encountered. 0·1 c.c. of a broth culture inoculated subcutaneously killed a guinea-pig in less than 24 hours, and the same bacillus was recovered from the animal's heart blood.

Specimen No. 32.

This portion of muscle was received at the laboratory two days after the animal's death. The report stated that the subcutaneous tissue, the muscular tissue, the stomach, the intestines, and the lungs were affected. The appearance of the muscle and the microscopical and cultural examinations were the same as those reported for Specimen No. 29, and 0·1 c.c. of broth culture inoculated subcutaneously proved lethal for a guinea-pig.

Specimen No. 33.

The muscle was received at the laboratory two days after the animal's death. The owner reported that he had not seen the sheep from which the specimen came, but his shepherd had said that no obviously diseased areas were seen in the carcase. Unfortunately, records of the appearance of the specimen when it was received at the laboratory were not kept. Cultures revealed ærobic contaminating bacteria, and *B. paludis* was not recovered by direct cultural means, but the bacillus was isolated from the heart blood of a guinea-pig inoculated with 1 c.c. of a saline emulsion of the muscular tissue and found dead on the morning following inoculation.

Specimen No. 34.

This specimen was received four days after the animal's death. The owner reported that the subcutaneous tissues, the muscular tissues, the stomach, the intestines, and the lungs were all affected. When the portion

of muscle was received, it was dark red in colour, moist, and inflamed ; a sourish smell was noted. Smears showed Gram-positive bacilli similar to those met with in Specimen No. 14, and *B. paludis* was recovered from the specimen in pure culture, no other bacteria being present.

Specimen No. 38.

This was received at the laboratory two days after the sheep had been found dead. The owner reported that lesions were present in the subcutaneous and muscular tissues of the neck and back, and that the stomach and intestines and lungs were affected. Upon receipt the muscle appeared red in colour, but apart from that there was no obvious abnormality. Smears showed bacilli similar to those from Specimen No. 14, and *B. paludis* was recovered in pure culture, no other bacteria being present. 0·1 c.c. of broth culture inoculated subcutaneously into a guinea-pig resulted in the animal being found dead 17 hours later. Cultures from the heart blood of this guinea-pig remained sterile.

Specimen No. 40.

This came from a sheep that had died three days previous to the receipt of the specimen. The owner reported that the subcutaneous and muscular tissues were affected. The muscle was moister than normal and deep salmon-pink in colour. Smears revealed a picture similar to that described for smears from Specimen No. 14. Cultures showed that *B. paludis* was not present in a pure state, but pure cultures were eventually obtained by direct cultural methods, and 0·1 c.c. of a broth culture proved lethal in 20 hours for an inoculated guinea-pig. Cultures from the heart blood of this animal showed bacilli similar to those used in the inoculation.

Specimen No. 47.

The specimen was received at the laboratory the day following the animal's death. The shepherd reported that lesions were present in the musculature of the throat and breast, and that the lungs were affected. The portion of muscle received was normal in appearance, but smears showed bacilli similar to those described from Specimen No. 14. *B. paludis* and *V. septique* were recovered by direct cultural methods. They appeared to be the only bacteria present. 0·1 c.c. of a broth culture of the *B. paludis* strain was inoculated subcutaneously into a guinea-pig, and the animal was found dead 18 hours later. The heart blood of this guinea-pig was sown into appropriate media, but no growth was obtained.

Specimen No. 49.

The sample of muscle was received at the laboratory the day after the animal's death. The owner reported that lesions were present under the skin. The muscle and the associated fat appeared deep pink in colour and excessively moist. The specimen had a slightly unpleasant and sourish smell. Smears showed numerous bacilli similar to those described from Specimen No. 14. By direct cultural means pure cultures of *B. paludis* and *V. septique* were obtained. A third type of colony growth was also observed, but pure cultures of the bacillus producing these colonies were not recovered by direct cultivation. A mixed culture, however, in 0·5 c.c. amount was inoculated subcutaneously into a guinea-pig. This animal died with a typical *B. chauvœi* infection, and *B. chauvœi* was isolated from its heart blood.

Specimen No. 50.

A portion of muscle was received at the laboratory two days after the animal's death. It was deep pink in colour and softer and more friable than normal. Smears presented a similar picture to those obtained from Specimen No. 14. *B. paludis* was recovered from the muscle in what appeared to be pure culture, and 0·5 c.c. was inoculated subcutaneously into a guinea-pig. The animal died in 48 hours, and *B. paludis* and *V. septique* were both recovered from its heart blood. 0·1 c.c. of a pure culture of the *B. paludis* strain isolated from the guinea-pig was inoculated into a second guinea-pig, and this animal was found dead the following day, and *B. paludis* was recovered from its heart blood in pure culture.

A guinea-pig inoculated subcutaneously with 1 c.c. of a normal saline emulsion of the sheep's muscle died in 24 hours. Post-mortem examination failed to show any sign of *V. septique* infection, but the lesions and the examination of smears made from the peritoneal fluid indicated that death was due to *B. paludis*. Culture media inoculated with the heart blood of this guinea-pig remained sterile.

Specimen No. 52.

This consisted of two pieces of muscle which came from a ewe that had lambed three weeks previously. The specimens were received the day after the animal was found dead. Both pieces of muscle were deep pink in colour, but one more so than the other. Smears were made from both pieces of muscle. Those from the dark coloured portion failed to show any bacteria, but smears from the remaining portion showed large numbers of bacilli similar to those described from Specimen No. 14, and cultures from this portion of muscle gave a pure growth of *B. paludis*. 0·1 c.c. of a broth culture was inoculated subcutaneously into a guinea-pig, and the animal was found dead 18 hours later. Cultures from the heart blood of this guinea-pig were not made, but it is of interest to note that a guinea-pig inoculated with 1 c.c. of a saline emulsion of the sheep's muscle died ; the post-mortem examination revealed a typical *B. paludis* infection, but cultures from the animal's heart blood remained sterile.

Specimen No. 75.

No particulars were received with this specimen, which consisted of a portion of muscle, œdematous and hæmorrhagic in appearance and of a sour offensive odour. Smears showed a large number of bacilli, similar to those present in smears from Specimen No. 14, but a larger sporulating bacillus was also present though in much smaller numbers. Cultures showed that a variety of ærobic contaminating bacteria were present, and pure cultures of *B. paludis* were not obtained by direct cultural means. A colony of *B. paludis* type, however, was sown into a tube of broth minced meat medium, and 0·2 c.c. of the resulting growth was inoculated subcutaneously into a guinea-pig. The animal died, and from its heart blood *B. paludis* was recovered.

From the above descriptions it will be seen that in the majority of cases the muscular tissue was abnormal in appearance and obviously diseased. In many cases *B. paludis* was the only micro-organism present, though in others it has been found associated with *V. septique*, and once apparently with *V. septique* and *B. chauvœi*, but in these cases an examination of smears made from the muscular tissues failed to reveal any evidence of infection with either *V. septique* or *B. chauvœi*.

When ærobic bacteria were present they were regarded as extraneous contaminating bacteria. In the case of Specimen No. 75 a large sporulating type of anærobic bacterium was present in the muscle. This was not isolated, but the specimen was not fresh, and in all probability the sporulating bacillus was a post-mortem invader.

The great number of Gram-positive, thick, stumpy, and relatively large bacilli which were present in all the specimens were identical in their appearance with the vegetative forms of *B. welchii*, and presented a picture similar to that found in smears made from the lesions in the muscular tissue of a sheep caused by *B. welchii* infection. (McEwen, 1930).

The Pathogenicity of *B. paludis*.

The pathogenicity of the majority of the strains was tested by inoculating guinea-pigs subcutaneously with small quantities of broth culture. No attempt was made to ascertain the minimum lethal dose, but when 0·1 c.c. of culture was inoculated it invariably proved fatal. Amounts of less than 0·1 c.c. were not tested.

The lesions shown in animals succumbing to inoculation of culture consisted of an extensive necrosis of the subcutaneous and muscular tissues. The skin over the affected parts became moist through the exudation of fluid, and the hair was easily detached. In the subcutaneous tissues there was a quantity of liquid material which could be detected as a fluctuating mass before incision of the skin was made. This liquid material was of a dirty greyish-pink colour, turbid in appearance, and contained fragments of necrotic tissue. The underlying muscles were pale and extensively necrosed and autolysed, and frequently a loop of intestine protruded through a hernia in the necrotic abdominal wall. The subcutaneous tissues of parts more distant from the seat of inoculation, *e.g.*, the axillæ and the under surface of the neck, were infiltrated with a pink, coagulated, gelatinous material from which, on cutting, fluid exuded. The abdominal cavity presented a picture of generalised congestion, the blood vessels being markedly injected. The stomach, intestines, and adrenals were congested. Smears made from the surface of the peritoneum showed vegetative forms of *B. paludis*, ranging from single rods to short chains of four individual bacilli. Long chains were never seen. The number of bacteria in peritoneal smears varied greatly from case to case. In animals with a hernia of the abdominal muscles the bacteria were present in great numbers, but in other cases they were comparatively scanty, only one or two being found in each field. Cultures from the heart blood were in some instances positive, but in others no growth occurred.

B. paludis, on one occasion only, has been inoculated into a sheep. The particular sheep used was hyperimmunised to *V. septique* infection and resisted large inoculations of that micro-organism without showing any ill effects. 0·5 c.c. of a broth culture of *B. paludis* was inoculated intramuscularly into the thigh. Fifteen hours later the animal was very lame, and it died at the forty-second hour. The inoculated leg

was greatly swollen from the thigh to the hoof. The skin on the inside of the thigh in the neighbourhood of the seat of the inoculation was purple in colour. The subcutaneous tissues of the leg and of the inguinal region were infiltrated and distended with a pale yellow, and in parts slightly blood-tinted, gelatinous material from which on incision fluid in large quantities escaped. Over the leg this infiltration reached a thickness of 2 to 5 cm., but in the region of the groin it was even greater The vessels in the subcutaneous tissues were injected and conspicuous. The musculature of the inoculated leg was pale, except for some reddened areas in the thigh, and sections through these showed that the red discoloration was due to intramuscular hæmorrhages situated around the more peripheral parts of the muscles. The intramuscular septæ were infiltrated with material similar to that encountered in the subcutaneous tissues. On opening the abdominal cavity the omentum presented an appearance of patchy inflammation due to congestion of the small blood vessels. The abomasum showed acute inflammatory areas in the mucosa, particularly in the neighbourhood of the greater curvature. The first few inches of the small intestine were acutely congested, but the remainder appeared to be normal. The large intestine was congested. The spleen presented numerous small subcapsular hæmorrhages. The liver, kidneys, and adrenals appeared to be normal. The thoracic cavity and the organs therein appeared normal. Smears were made from the fluid in the subcutaneous tissues and from the surface of the peritoneum, but no bacteria were found in them. Smears from the muscle of the inoculated thigh showed a few bacilli only. Cultures were made from the heart blood into suitable media, but these remained sterile.

The evidence which has been summarised above shows *B. paludis* to be a pathogenic bacterium capable of producing a fatal disease of sheep in the field, which disease may be reproduced experimentally in sheep by the inoculation of cultures of the bacilli. The disease is characterised by an acute inflammatory reaction in parts of the muscular and subcutaneous tissues of the body, the bacteria being present in these areas and generally in great numbers. The stomach and intestines may be involved and show congestion of their walls. *B. paludis* is commonly the only micro-organism present in the esions in the muscular tissue, but it has also been found in association with other pathogenic anærobic bacteria. Microscopical examinat ons of smears made from the muscular tissue, however, have not indicated the presence of these other pathogenic bacteria, and the evidence points to *B. paludis* as being the primary cause of death in all of the twelve cases here reported.

Morphological and Cultural Characters of *B. paludis*.

The twelve strains of *B. paludis* considered in this paper were all obtained in pure culture before details of their characters were considered. Pure cultures were secured by repeatedly subculturing, alternately on serum agar slopes and in broth containing minced meat.

Individual colonies on serum agar were sown each into a separate tube of broth minced meat medium, and growth from this medium was in turn seeded on to serum agar. The process was repeated until the purity of the strain was satisfactorily established.

Morphology.

Bacilli from 24 hours' broth minced meat medium appeared as simple vegetative rods, single or in pairs. The bacilli were often collected into small bundles, the individual bacilli being in parallel formation. The bacilli were stout and stumpy, with bluntly rounded or almost square cut ends, in shape and size closely resembling the well-known *B. welchii.* Spores were not seen in smears made from cultures in this medium ; but from alkaline egg medium and cultures on solid serum sporulating forms were observed, the spores being oval and of greater width than the bacillus, and either central or subterminal in position. Citron or spindle-shaped forms of the bacteria were not noticed.

The bacilli stained intensely with ordinary aniline dyes and the great majority in young broth minced meat cultures were strongly Gram-positive, though a number showed irregularities in their retention of the stain, the commonest being an unstained area running the length of the bacillus. A few bacilli were entirely Gram-negative.

Bacilli from broth minced meat cultures were examined for the presence of a capsule, but capsule formation was not demonstrated. Bacilli, however, from animal tissue and fluids were not submitted to this examination, and the question of capsule formation has yet to be definitely settled.

Motility.

Sixteen and twenty-hours' broth minced meat cultures were tested for motility by placing a small quantity of the culture on a slide, covering with a coverslip, ringing round the edge of the cover slip with warm liquid vaseline, and examining under the No. 6 objective. Examined in this manner, the great mass of the bacilli were totally without motion, but occasionally bacilli appeared to perform slow tumbling and turning movements, and to be sluggishly motile. The slight degree of motility exhibited was too indefinite to be of any critical value, and was such that independent observers differed among themselves in their interpretation as to whether motility was present or absent.

Examinations of *B. paludis* under dark ground illumination have not revealed the presence of flagella, which suggests that the sluggish motility of certain individuals observed when the No. 6 objective was used was not due to true bacterial motility but to some other factor.

Cultural Characters.

In all instances cultures were maintained at a temperature of 37° C.

The bacilli were anærobic ; no growth occurred either in liquid or on solid media under ærobic conditions.

The McIntosh and Fildes electrically warmed anærobic jar was used to secure anærobiasis for surface growths, and in the case of liquid media free O_2 was excluded by covering the surface of the liquid with a layer of vaseline before sterilisation of the media. All inoculations of liquid media were made with a Pasteur pipette through the vaseline seal previously liquefied by warming. The volume of the gas evolved in liquid media was estimated roughly by noting the extent of the upward displacement of the vaseline seal.

Serum agar. Surface colonies varied considerably in size. The smaller colonies were 1 to 2 m.m. in diameter. These were simple round, convex, smooth colonies, greyish-white in colour, opaque, with regular free margins, and raised upon the surface of the medium. Larger colonies up to 5 or 6 m.m. across had a similar colour and consistency. They were, however, more spreading in character and relatively flatter, and in many instances their free border was irregular, presenting blunt indentations, and running from each indentation towards the centre of the colony low ridges were sometimes observed under the hand lens ; these ridges faded out before the centre of the colony was reached. The most central part of the colony was either flat or slightly raised.

Blood agar. The colonies were similar to those descr bed on serum agar except that on this medium they appeared less opaque and were surrounded by a wide zone of hæmolysis.

Shake agar. Deep colonies were opaque and lenticular and up to 2 m.m. in diameter.

Shake blood agar. Deep colonies were similar to those produced in shake agar, and were surrounded by a clear zone of *beta* hæmolysis.

Broth containing minced meat. Vigorous growth occurred at the end of 24 hours, with the evolution of gas and the production of a moderate turbidity of the medium. At three days there was an increased pink coloration of the meat, and after one week the meat was changed to a deep pink colour, and there was considerable disintegration of the meat particles, but no distinct digestion. Incubation for periods of four to five weeks produced no further change. Blackening of the meat or the formation of black pigment under the vaseline seal was never noted.

Broth. After 24 hours there was evolution of gas and the medium was uniformly turbid. On the third day the turbidity showed signs of clearing, and at the end of one week the medium was clear, and a small white compact deposit had settled out in the bottom o the tube.

Broth containing a small portion of brain tissue and a piece of iron wire. After a week's incubation in this medium blackening was observed under the vaseline seal and on the sides of the tube beneath the seal and above the level of the broth. The brain tissue sometimes showed traces of blackening. No further change was produced by longer incubation.

Skim milk. At the end of 24 hours there was vigorous gas production, and a large greatly fragmented clot was formed (typical "stormy" clot reaction). At the end of three days the clot was reduced in size, and after one week a spongy shrunken clot remained, reduced to approximately one-third of its original size. The expressed whey was clear. At the end of four weeks of incubation the culture assumed a faint buffy tint, but no further change was noted.

Skim milk containing a small portion of brain tissue. The growth in this medium was similar to that in skim milk.

Cream (1 *c.c. of washed, sterilised cream added to* 10 *c.c. of broth*). After three days' incubation there was no appreciable alteration in the appearance of the cream, but after one week's incubation the cream was converted to a flocculent greyish-white material. No further alteration was noted at the end of four or five weeks' incubation.

Alkaline egg medium. This medium was rendered turbid, but no coagulum or large flocculi were produced.

12½ *Gelatine in broth.* When cultures were removed from the incubator they were placed in the ice chest for an appropriate period, and then examined for liquefaction. Uninoculated tubes were treated in a like manner and acted as controls. Gelatine was liquefied in 24 hours.

Coagulated egg white cubes in broth. No alterations were noted in the character of the egg white until incubation had been continued for three to four weeks, when a darkening in colour and an increased transparency of the cubes were produced. At the end of five weeks' incubation the cubes were of a clear deep brown colour towards their centres, while the edges were clear and transparent.

Inspissated serum slopes. After four weeks' growth slight erosion of the serum at the lower part of the slopes was noted. Incubation for a period of eight weeks produced distinct but not extensive liquefaction of the medium with accumulation of fluid.

Fibrin. (*Small piece of fibrin in a tube of broth.*) No evidence of any digestion or alteration of the fibrin was observed even after prolonged incubation.

Fermentation reactions, acid and gas production being taken as indications of fermentation. The basal medium consisted of peptone water containing a small cube of brain tissue. The materials to be tested were sterilised separately by autoclaving, and a sufficient quantity of a 10 per cent. solution was added to the basal medium to make the final concentration of the test substance 1 per cent. The *p*H of the finished medium was remarkably constant for each batch produced, but different batches of media varied in their *p*H from 6·5 to 7·0. Fermentation was considered to have occurred when the *p*H of the medium was reduced by 0·5 or more and when a volume of gas at least equal to half the volume of the culture fluid was produced. The *p*H of the medium was read after one week's incubation and again after four weeks' incubation by the colorimetric method, small quantities of fluid (0·5 c.c.) being tested and matched against buffer solutions of known *p*H. The two readings were very

constant for all the substances tested except salicin, which in some instances gave a markedly lower *p*H reading at the second test.

Glucose, lactose, levulose, galactose, maltose, saccharose, and glycerine were fermented by all the strains, and with the exception of Strain 32 all the strains fermented salicin. Glycerine and salicin did not give rise to such an abundant volume of gas nor to such an extensive drop in the *p*H as did the fermentable sugars. Inulin and mannite were not fermented. The utilisation by the bacilli of 0·2 per cent. of glucose, lactose, levulose, galactose, maltose, and saccharose in the basal medium, was checked by testing for the presence of reducing sugars in the media after one week's incubation by the Benedict qualitative method, the technique described by Brown (1925) being adopted. In no instance was any sugar detectable after one week's growth, and these results confirm the fermentation reactions recorded for the same sugars when acid and gas productions were the criteria of fermentation. Before applying the Benedict test when saccharose was used the sugar was converted to a reducing sugar by boiling the sample of the medium to be tested with dilute HCl.

Comparison of the Morphology, Cultural Characters, and Virulence of *B. paludis* and *B. welchii*.

Morphology.

In smears stained by ordinary methods both the vegetative and sporulating forms of *B. paludis* and *B. welchii* are very much alike, and it is not possible to distinguish one from the other. No capsule has been demonstrated for *B. paludis*, but absence of capsule cannot at present be taken as a differential character distinguishing the one type of bacillus from the other, because cultures of *B. welchii* were examined for capsule formation under comparable conditions and none was found, although Robertson (1916) states that *B. welchii* is invariably a capsulated bacillus and that the capsule may be seen in cultures examined in weak alkali solution or serum.

The motility of *B. paludis* is very doubtful, but, even assuming true bacterial motility to be present, the degree of motility demonstrable by the technique employed is not sufficient to make it either an easy or reliable method of distinguishing *B. paludis* from the non-motile *B. welchii*.

Cultural Characters.

In their cultural characters *B. paludis* and *B. welchii* closely resemble one another.

The shape and form of colonies, the stormy clotting of milk, the absence of coagulation in alkaline egg medium, and the liquefaction of gelatine in cultures of *B. paludis*, agree with the accepted cultural characters of *B. welchii*. The *beta* type of hæmolysis produced by *B. paludis* and the formation of a flocculent material from cream are in agreement with the reactions of *B. welchii* described by Brown (1925), and the production of black pigment in media containing

iron by *B. paludis* is similar to the reaction produced by *B. welchii* under comparable conditions (Hall, 1922 ; Kahn, 1925). But when the action of the two bacilli over long periods of time upon coagulated egg white and solid serum is considered, differences in behaviour become apparent. *B. paludis* darkens egg white, and causes it to become transparent, and solid serum is slowly liquefied. This bacillus is therefore more proteolytic than *B. welchii*, which does not attack or alter these proteins (Brown, 1925).

The fermentation reactions of the two bacilli show much in common, both fermenting a wide range of substances, including glucose, lactose, levulose, saccharose, galactose, and maltose.

All the twelve strains of *B. paludis* fermented glycerine, but none fermented inulin. On these fermentations of glycerine and inulin *B. paludis* cannot be differentiated from *B. welchii*, as strains of *B. welchii* vary in their capacity to ferment glycerine and inulin, some strains fermenting either the one or the other, while yet other strains ferment both substances. It is, however, agreed that *B. welchii* does not ferment salicin. Salicin was fermented by eleven of the twelve strains of *B. paludis* studied, and in this respect the eleven strains differ from *B. welchii*.

Virulence. According to the majority of authors cited by Robertson (1929) and O'Brien (1929), *B. welchii* is pathogenic for guinea-pigs in amounts of 0·2 c.c. to 1 c.c. inoculated intramuscularly. *B. paludis* has invariably been found pathogenic for guinea-pigs when 0·1 c.c. of a culture of broth minced meat medium has been inoculated subcutaneously. This small amount of culture produced extensive lesions and death in 24 hours or less. Smaller quantities of culture would, in all probability, have proved fatal for guinea-pigs, but these were not tested. Up to the present there has been no appreciable loss of virulence in strains kept in artificial culture for several months. *B. paludis* would therefore appear to be a more virulent and more highly pathogenic bacterium than *B. welchii*, but at present this greater virulence must be regarded as one of degree only, and not as a reliable means of distinguisling *B. paludis* from *B. welchii*, though it may serve as an indication of which micro-organism is being dealt with.

The lesions produced in guinea-pigs by *B. paludis* and *B. welchii* are similar.

Cultures of *B. paludis* have not been inoculated into mice, but small quantities of bacteria-free filtrates have frequently been inoculated into these animals with fatal results. In no instance has hæmoglobinuria ever been observed in mice dying from inoculation with *B. paludis* filtrates, although this particular feature has been observed frequently in mice inoculated with *B. welchii* filtrate, and is a well recognised, though not constant, post-mortem characteristic of this condition. It may therefore be concluded that *B. paludis* intoxication does not give rise to a hæmoglobinuria, and in this respect differs from intoxication with *B. welchii*. Further, the toxin of *B. paludis* is distinct and different from that of *B. welchii*, but this subject is discussed at greater length later on.

Another bacillus which might appear similar to *B. paludis* is the " lamb dysentery bacillus," described by Dalling (1928), but the characters of this bacillus, as described by that author, which distinguish it from *B. welchii* serve equally well to distinguish the " lamb dysentery bacillus " from *B. paludis*, as, for example, the active proteolytic properties of the " lamb dysentery bacillus," which enable it rapidly to liquefy solid serum, its coagulation of alkaline egg medium, and its inability to ferment glycerine, are all features which are not shared by *B. paludis* and should prevent confusion arising between the two species.

B. paludis Toxin.

Cultures were obtained in diffusion shell 1 per cent. glucose broth medium *p*H 7·4 (McEwen, 1926), and after 24 hours' incubation the rich growths were centrifuged until they were clear ; the clear fluids were then filtered through Seitz filters and the filtrates tested for sterility. The toxicity of the sterile filtrates from the twelve strains was tested by the inoculation of laboratory animals.

In all twelve instances small quantities of filtrate inoculated intravenously into rabbits caused death in a few minutes ; and those filtrates which were tested by the subcutaneous inoculation of rabbits and guinea-pigs, or by the intramuscular inoculation of mice, produced an extensive œdema and necrosis and, if sufficient filtrate were inoculated, the death of the animals.

Table No. 1 shows the result of inoculating varying quantities of filtrate intravenously into rabbits.

TABLE I.

Rabbit.	*Weight.*	*Amount of filtrate inoculated intravenously (Filtrate from Srain 52).*	*Result.*
1	2500 grams	2 c.c.	Dead in 3 minutes
2	2200 grams	1 c.c.	Dead in 7 minutes
3	2500 grams	0·5 c.c.	Dead in 7 minutes
4	2150 grams	0·25 c.c.	Died overnight
5	2000 grams	0·125 c.c.	Died overnight
6	1900 grams	0·0625 c.c.	Lived
7	2050 grams	0·03125 c.c.	Lived

TABLE II.

Records the Result of the Intramuscular Inoculation of Mice with Varying Quantities of Filtrate.

Mice.	*Amount of filtrate inoculated intramuscularly (Filtrate from Strain 52).*	*Result.*
1 & 2	0·15 c.c.	Both dead in less than 24 hours
3 & 4	1 c.c.	Both dead in less than 24 hours
5 & 6	0·05 c.c.	One dead in less than 48 hours. The other survived the 48th hour but inoculated leg was swollen and paralysed.
7 & 8	0·02 c.c.	Survived but in both ; the inoculated leg was swollen and paralysed.
9 & 10	0·004 c.c.	One had a slightly swollen leg. The other remained normal.

The Effect of Heat Upon Toxic Filtrate.

Filtrate from Strain 52 was heated in the water bath for periods of 30 and 60 minutes at a temperature of 60° C. Animals inoculated with the heated filtrate remained normal. The results of these inoculations are shown in Table III. The effect of temperatures lower than 60° C. upon toxic filtrate, or of a temperature of 60° C. acting for a period of less than 30 minutes, has not been studied.

TABLE III.

Rabbit.	*Weight.*	*Amount of filtrate inoculated into each animal.*	*Result.*
8	1950 grams	1 c.c. of filtrate heated to 60° C. for 60 minutes, inoculated intravenously	Lived
9	1950 grams	1 c.c. of filtrate heated to 60° C. for 30 minutes, inoculated intravenously	Lived
Mice			
11 & 12		0·15 c.c. of filtrate heated to 60° C. for 60 minutes, inoculated intramuscularly	Remained normal
13 & 14		0·15 c.c. of filtrate heated to 60° C. for 30 minutes, inoculated intramuscularly	Remained normal

The Effect of Storage at Low Temperature upon Toxic Filtrate contained in a hermetically sealed tube and in a tube plugged with porous cotton wool.

Filtrates were stored in the refrigerator at a temperature of approximately 2° C. for eight weeks, but at the end of this period no appreciable diminution in toxicity was detected ; filtrates from the hermetically sealed tube and the tube plugged with cotton wool both killed rabbits a few minutes after intravenous inoculations were made.

Immunisation Against Toxic Filtrates of B. paludis.

Filtrate from Strain 32 in 0·5 c.c. amount inoculated intravenously into a rabbit of 2,500 gms., killed the animal in 10 minutes. The broth culture, from a portion of which this filtrate had been made, was divided into two parts and to one 0·8 per cent. of formalin, and to the other 0·4 per cent. of formalin, was added. The formalinised cultures were then incubated at 37° C. for 48 hours and stored in the refrigerator.

Four rabbits—No. 13, 2,100 gms. ; No. 14, 2,500 gms. ; No. 15, 2,700 gms. ; and No. 16, 2,500 gms., were inoculated intravenously with 1 c.c. of the supernatant fluid collected after centrifuging that portion of culture containing 0·8 per cent. formalin, and two days later they again each received another intravenous inoculation of 3 c.c. No symptoms were produced by the inoculations, and later the immunisation was continued by the intravenous inoculations of the supernatant fluid from the portion of culture treated with 0·4 per cent. formalin. This treatment was finally followed up by the subcutaneous inoculation of small amounts of fresh toxic filtrate without formalin.

At the end of the period of immunisation Rabbit No. 15 was inoculated intravenously with 1·5 c.c. of filtrate 32. This animal

remained well, but a control rabbit, No. 17, of 2,600 gms., inoculated at the same time and with a similar amount of the filtrate, died in four minutes. Rabbits Nos. 13, 14, and 16 were each inoculated subcutaneously with 3 c.c. of filtrate 32. No lesion resulted from the inoculation, but of the control rabbits Nos. 18 and 19, of 2,500 gms. each, which were each inoculated with the same amount of filtrate, No. 18 was found dead the following morning, and post-mortem examination revealed great infiltration of the subcutaneous tissues with a gelatinous, semi-liquid, pale straw-coloured material, and hæmorrhages in the underlying musculature ; while No. 19 was dull, the inoculated leg being greatly swollen and the skin purple in colour. On the second day it was considered necessary to chloroform the animal, and the post-mortem examination showed a picture similar to that described for rabbit No. 18.

From these experiments it is clear that in rabbits Nos. 13, 14, 15, and 16 an active immunity against the toxic filtrates of *B. paludis* had been produced.

Passive Immunity to the Toxic Filtrates of B. paludis.

Rabbits Nos. 13, 14, 15, and 16, after immunisation, were bled, and the serum from each animal was tested for antibodies which would neutralise the toxic action of filtrates. Sera from animals Nos. 14 and 16 appeared to be the more potent, and these sera were pooled, and used in the tests recorded below.

Two guinea-pigs were each inoculated subcutaneously with 3 c.c. of *B. paludis* antiserum. Two days later these and two control guinea-pigs each received a subcutaneous inoculation of 0·5 c.c. of filtrate from Strain No. 29. The day following the inoculation of the filtrate both of the control guinea-pigs had extensive fluctuating œdematous swellings at the seat of the inoculation. One of the guinea-pigs treated with serum showed a small circumscribed œdematous swelling, while the other animal treated with serum appeared normal. On the third day the control guinea-pigs had very extensive œdematous swellings, but the animals which had received serum had the merest trace of swelling at the point where the inoculation of the filtrate was made. On the fifth day the skin over the affected parts of the control animals was moist, necrotic, and denuded of hair, while the serum-treated animals showed small areas $\frac{1}{4}$-in. in diameter covered by a dry scab. The extensive lesions in the control animals necessitated their being chloroformed, but the other animals remained bright, and rapidly lost all evidence of having been inoculated.

The results of the above experiment showed that the inoculation with the immune serum gave the guinea-pigs a distinct protection against the toxic principal in the filtrate.

The Specificity of the Toxic Filtrates of B. paludis.

As a close resemblance exists between *B. paludis* and *B. welchii* it was deemed essential to study the action of *B. welchii* antiserum

upon the toxic filtrate of *B. paludis*. This was particularly necessary as *B. welchii* has been recovered from the musculature of a sheep which presented a similar appearance to the specimens from which *B. paludis* has been isolated (McEwen, 1930). Further, *B. welchii* has been reported by Meissner and Albrecht (1924) and by Karmann and Seifreid (1924) as the occasional cause of black-quarter or gas œdema in sheep, but these authors apparently arrived at a diagnosis without the aid of neutralisation tests of toxin or virulent culture by specific immune serum.

Through the kindness of Mr. J. H. Mason, F.R.C.V.S., of the Wellcome Physiological Laboratories, a supply of *B. welchii* antiserum was received, and parallel tests with mixtures of *B. paludis* filtrate and its specific antiserum were conducted along with mixtures of *B. paludis* filtrate and *B. welchii* antiserum. The mixtures were allowed to stand at room temperature for one hour before the inoculations were made. These tests are recorded in Table No. VI. The toxic filtrates from each of the twelve strains of *B. paludis* were neutralised by the *B. paludis* antiserum, but were not affected by the *B. welchii* antiserum.

At this stage of the work attempts were made to obtain a potent *B. welchii* toxin in the hope of testing the action of *B. paludis* antiserum upon *B. welchii* toxin, but a potent *B. welchii* toxin was not obtained and, accordingly, the possibility of *B. paludis* antiserum neutralising the pathogenic action of cultures of *B. welchii* was investigated, and

TABLE VI.

Intramuscular Inoculation of Mice.

Mouse No.	*L.D. filtrate.*	B. paludis *antiserum.*	B. welchii *antiserum.*	*Result.*
		Toxic Filtrate No. 32 *L.D.* 0·2 *c.c.*		
15 & 16	0·2 c.c.	—	—	Both dead in 24 hours
17 & 18	0·2 c.c.	0·1 c.c.	—	Lived. No symptoms
19 & 20	0·2 c.c.	0·05 c.c.	—	Swelling of inoculated leg. Both survived
21 & 22	0·2 c.c.	—	0·1 c.c.	Both dead in 24 hours
23 & 24	0·2 c.c.	—	0·05 c.c.	Both dead in 24 hours
		Toxic Filtrate No. 47. *L.D.* 0·15 *c.c.*		
25 & 26	0·15 c.c.	—	—	Both dead in 24 hours
27 & 28	0·15 c.c.	0·1 c.c.	—	Swelling of inoculated leg. Both animals lived
29 & 30	0·15 c.c.	0·02 c.c.	—	Both dead in 24 hours
31 & 32	0·15 c.c.	—	0·2 c.c.	Both dead in 24 hours
33 & 34	0·15 c.c.	plus 0·1 c.c. normal rabbit serum		Both dead in 24 hours
		Toxic filtrate No. 52. *L.D.* 0.15 *c.c.*		
35 & 36	0·15 c.c.	—	—	Both dead in 24 hours
36 & 37	0·15 c.c.	0·1 c.c.	—	Inoculated leg swollen. Both lived
38 & 39	0·15 c.c.	—	0·2 c.c.	Both dead in 24 hours
40 & 41	0·15 c.c.	plus 0·1 c.c. normal rabbit serum		Both dead in 24 hours

TABLE VI.—*Continued.*

INTRAVENOUS INOCULATIONS OF RABBITS.

Rabbit No.	*Weight.*	*L.D. of filtrate.*	B. paludis *antiserum.*	B. welchii *antiserum.*	*Result.*
			Toxic Filtrate No. 52.		
23	2200 gms.	1 c.c.	—	—	Died in 7 minutes
24	2050 gms.	1 c.c.	1 c.c.	—	Lived
25	2500 gms.	1 c.c.	0·5 c.c.	—	Lived some hours. Died over night
26	2200 gms.	1 c.c.	0·25 c.c.	—	Died in 12 minutes
27	2400 gms.	1 c.c.	—	1 c.c.	Died in 3½ minutes
28	2000 gms.	1 c.c.	plus 1 c.c. normal rabbit serum		Died in 3½ minutes
			Toxic Filtrate No. 14.		
29	2200 gms.	0·5 c.c.	—	—	Died in 6 minutes
30	1500 gms.	0·5 c.c.	0·5 c.c.	—	Lived
31	2400 gms.	0·5 c.c.	—	1 c.c.	Died in 4 minutes
			Toxic Filtrate No. 29.		
32	2200 gms.	0·5 c.c.	—	—	Died in 4 minutes
33	2000 gms.	0·5 c.c.	0·5 c.c.	—	Lived
34	2000 gms.	0·5 c.c.	—	1 c.c.	Died in 4 minutes
			Toxic Filtrate No. 33.		
35	2200 gms.	0·5 c.c.	—	—	Died in 10 minutes
36	2050 gms.	0·5 c.c.	0·5 c.c.	—	Lived
37	1500 gms.	0·5 c.c.	—	1 c.c.	Died in 7 minutes
			Toxic Filtrate 34.		
38	1850 gms.	0·25 c.c.	—	—	Died in 8 minutes
39	2200 gms.	0·25 c.c.	0·5 c.c.	—	Lived
40	2500 gms.	0·25 c.c.	—	1 c.c.	Died in 10 minutes
			Toxic Filtrate No. 38.		
41	2300 gms.	0·5 c.c.	—	—	Died in 14 minutes
42	2000 gms.	0·5 c.c.	0·5 c.c.	—	Lived
43	2200 gms.	0·5 c.c.	—	1 c.c.	Died in 30 minutes
			Toxic Filtrate No. 40.		
44	2000 gms.	0·5 c.c.	—	—	Died in 16 minutes
45	2400 gms.	0·5 c.c.	0·5 c.c.	—	Lived
46	1850 gms.	0·5 c.c.	0·5 c.c.	—	Lived
47	2250 gms.	0·5 c.c.	—	1 c.c.	Muscular weakness in 30 minutes, but animal appeared to recover ; died on 3rd day
48	1700 gms.	0·5 c.c.	—	1 c.c.	Died in 20 minutes
			Toxic Filtrate No. 49.		
49	2600 gms.	0·5 c.c.	—	—	Died in 17 minutes
50	1950 gms.	0·5 c.c.	0·5 c.c.	—	Lived
51	2200 gms.	0·5 c.c.	—	1 c.c.	Died in 25 minutes
			Toxic Filtrate No. 50.		
52	2000 gms.	0·5 c.c.	—	—	Died in 6 minutes
53	2800 gms.	0·5 c.c.	0·5 c.c.	—	Lived
54	2300 gms.	0·5 c.c.	—	1 c.c.	Died in 13 minutes
			Toxic Filtrate No. 75.		
55	2700 gms.	0·5 c.c.	—	—	Died in 6 minutes
56	2000 gms.	0·5 c.c.	0·5 c.c.	—	Died in 30 minutes
57	2000 gms.	0·5 c.c.	—	1 c.c.	Died in 6 minutes
58	2000 gms.	0·5 c.c.	0·5 c.c.	—	Lived
59	2300 gms.	0·5 c.c.	—	1 c.c.	Died in 8 minutes

at the same time the capacity of the *B. welchii* antiserum to protect against the pathogenic action of cultures of *B. welchii* was tested.

It was found that *B. paludis* antiserum had no neutralising effect upon the pathogenicity of cultures of *B. welchii*, though the same cultures were rendered harmless for inoculated animals by the action of the *B. welchii* antiserum. These results are recorded in Table No. VII, and the combined results of the experiments enumerated in Tables Nos. VI and VII furnish proof that *B. paludis* is an independent and distinct species from *B. welchii*.

TABLE VII.

THE ACTION OF *B. paludis* ANTISERUM AND OF *B. welchii* ANTISERUM UPON BROTH CULTURES OF B. WELCHII.

Two Strains of B. welchii *were used, one was Strain No.* 273 *of the National Collection of Type Cultures, and the other was a strain isolated from the muscle of a sheep. The mixtures of serum and cultures were inoculated into the subcutaneous tissues.*

Strain.	*L.D. of culture*	*Guinea pig.*	B. paludis *antiserum.*	B. welchii *antiserum.*	*Result.*
273	0·25 c.c.	1	—	—	Died in 24 hours
273	0·25 c.c.	2	—	—	Died on third day
273	0·25 c.c.	3	0·75 c.c.	—	Died in 24 hours
273	0·25 c.c.	4	0·75 c.c.	—	Severe and extensive lesions. Chloroformed on third day
273	0·25 c.c.	5	—	0·25 c.c.	Remained well
273	0·25 c.c.	6	—	0·25 c.c.	Remained well
273	0·25 c.c.	7	—	0·1 c.c.	Remained well
273	0·25 c.c.	8	—	0·1 c.c.	Remained well
Isolated from a sheep	1 c.c.	9	—	—	Died in 16 hours
,,	1 c.c.	10	—	—	Died in 16 hours
,,	1 c.c.	11	0·75 c.c.	—	Died in 16 hours
,,	1 c.c.	12	0·75 c.c.	—	Died in 16 hours
,,	1 c.c.	13	—	0·5 c.c.	Remained well
,,	1 c.c.	14	—	0·5 c.c.	Remained well
,,	1 c.c.	15	—	0·1 c.c.	Severe local lesions, but recovered
,,	1 c.c.	16	—	0·1 c.c.	Severe local lesions, but recovered

The evidence also points to a distinction between *B. paludis* and the " lamb dysentery bacillus." The " lamb dysentery bacillus " shows in its antigenic complex an antigen common to itself and *B. welchii*, as the antiserum of the former is capable of neutralising the toxin of the latter although the reaction is not a reversible one (Darling, 1928). *B. paludis* antiserum, however, has no such property.

The rapidity of the lethal action of *B. paludis* filtrates when inoculated intravenously into rabbits resembles the action of *V. septique* toxin (Robertson, 1920) and *B. histolyticus* toxin (Weinberg and Seguin, 1917). Confusion of the identity of these micro-organisms should never arise on account of their marked dissimilarity in morphology and cultural reactions. Nevertheless, as *V. septique* had in a number of instances been isolated from material from which

B. paludis was recovered, it was deemed to be of sufficient interest to test the effect of inoculating animals with mixtures of *B. paludis* filtrate and *V. septique* antiserum. The *V. septique* antiserum was prepared by hyperimmunising a sheep with repeated inoculations of formalinised culture, and these were followed by inoculations of virulent culture. The serum from this animal, in appropriate amounts, completely neutralised quantities of *V. septique* toxin which was capable of killing rabbits in a few minutes, but the same serum had no neutralising effect upon the toxic filtrates of *B. paludis*.

The results of these experiments are given in Table No. VIII.

It is of interest to note that the sheep which supplied the *V. septique* antiserum was the same animal which succumbed in 42 hours to the inoculation of 0·5 c.c. of a culture of *B. paludis*.

TABLE VIII.

THE EFFECT OF *V. septique* ANTISERUM UPON *V. septique* TOXIN AND UPON *B. paludis* TOXIC FILTRATES.

Rabbit.	*Weight.*	V. septique *toxin.*	B. paludis *toxic filtrate.*	V. septique *antiserum.*	B. paludis *antiserum.*	*Result.*
60	2050 gms.	1·5 c.c.	—	—	—	Died in 5 minutes
61	2250 gms.	1·5 c.c.	—	1 c.c.	—	Lived
62	2400 gms.	1·5 c.c.	—	1 c.c.	—	Lived
63	2300 gms.	—	1·5 c.c.	1 c.c.	—	Died in 4 minutes
64	3600 gms.	—	1·5 c.c.	1 c.c.	—	Died in 6 minutes
65	1800 gms.	—	1·5 c.c.	—	1 c.c.	Lived several hours, died overnight

Summary of the Features presented by B. paludis *Toxic Filtrates.*

The intravenous inoculation of rabbits with *B. paludis* filtrate caused the death of these animals in a few minutes, and subcutaneous inoculations of filtrate into rabbits and guinea-pigs and the intramuscular inoculation of mice produced an extensive œdema and necrosis of the inoculated parts and the surrounding tissues, and if sufficient filtrate were inoculated the death of the animals.

Lysis of the red blood cells of inoculated animals has not been observed, and hæmolysis did not result from the incubation of mixtures of washed red blood cells in saline and toxic filtrate.

Rabbits immunised against the toxic filtrates showed a complete resistance to intravenous and subcutaneous inoculations of lethal doses of filtrate, and guinea-pigs inoculated with the serum of immune rabbits have exhibited a markedly increased resistance to the toxic action of subcutaneous inoculations of filtrate.

The toxicity of filtrates was completely neutralised by the action of immune serum *in vitro*.

The evidence concerning the action of formalin upon toxic filtrates, though incomplete, points to the formation of an atoxic antigenic substance.

The toxic principal in the filtrate was thermolabile.

From the foregoing characters exhibited by the toxic filtrates it is concluded that the toxic principle in the filtrates is a true bacterial toxin.

Toxins were easily obtained and have been prepared from strains kept in artificial cultivation for periods of from one to fourteen months. Further, the *p*H of the medium, within the limits which were tested, had little appreciable effect upon the production of toxin, toxic filtrates being obtained from media with a *p*H of 6·8, 7·2, 7·4 and 7·6.

The facility with which *B. paludis* toxin is obtained in filtrates is in contrast with the difficulty so frequently experienced in obtaining toxic filtrates of *B. welchii* (Robertson, 1929).

Symptoms and Post-mortem Appearance shown by Animals Inoculated with B. paludis *Toxin.*

The first rabbit to be inoculated intravenously with toxin received a large dose, between 4 c.c. and 5 c.c. The intention had been to test the effect of the inoculation of 5 c.c., but before the total amount had been inoculated the animal collapsed and died, it being a matter of seconds only from the commencement of the inoculation to the time of death. Smaller quantities of toxin cause death after a variable period ranging from a few minutes to several hours, depending upon the quantity of toxin inoculated. When death occurs a few minutes after inoculation symptoms of muscular weakness are first seen. The ears droop backwards and the head is gradually lowered upon the neck, the muscles of the limbs become flaccid, and the animal lies limply on the ground. During this time the respirations are shallow and more rapid than normal. Death may supervene quietly and peacefully, but more generally it is preceded by a short period of muscular excitability, irregular running or kicking movements being performed as the animal lies on the ground, then for a few seconds respiration cases, and the thorax remains distended, but immediately prior to death one or two gasping respiratory movements are performed. A few fæces are passed at the commencement of the period of muscular weakness. When the appearance of the muscular weakness is delayed for 10 to 20 minutes or longer the symptoms are not so severe ; the animals assume a crouching position and are unable or unwilling to move. Partial recovery takes place after the lapse of a short period, and then, except perhaps for a general dullness in looks and behaviour, the animals appear to be normal. However, they almost invariably die at the end of a few hours. Occasionally symptoms of muscular weakness are not shown, but the animals are found dead several hours after having been inoculated.

The symptoms produced by subcutaneous or intramuscular inoculations of toxin are those associated with the local œdema and

necrosis, but when an excess of toxin is inoculated symptoms of profound intoxication are shown, and death follows.

Post-mortem examinations of animals dying from intravenous inoculation have failed to reveal any noteworthy lesions. In animals inoculated subcutaneously or intramuscularly the lesions of œdema and necrosis only are present, and these are confined to the inoculated and adjacent parts.

General Discussion on the Recognition of *B. paludis*.

To distinguish *B. paludis* from *B. welchii* the most critical test, namely, the neutralisation of toxin or of the pathogenicity of culture by specific immune serum, should be employed. Now, in the identification of *B. welchii* great reliance has been placed upon morphology and cultural characters and serum neutralisation tests have been neglected in the majority of cases, and, when the close similarity of the morphology and cultural reactions of *B. paludis* and *B. welchii* are considered, it is reasonable to suppose that had *B. paludis* been encountered it might have been labelled *B. welchii*. Only within recent years has confusion regarding the identity of *B. chauvœi* and *V. septique* ceased to reign, and the distinguishing features of these two micro-organisms are much more marked and more easily detected than are the characters which distinguish *B. paludis* from *B. welchii*.

Considerable attention has been given to diseases of cattle due to the anærobic sporulating bacteria, but it is doubtful if *B. welchii* by itself produces disease in these animals. Noller and Seelemann (1924) consider that it may do so, but the evidence they bring forward is by no means conclusive. *B. welchii* has on occasion been isolated along with other pathogenic anærobic bacteria from material derived from cases of black-quarter—Warringholz and Rassfeld (1924), Zwick (1924), Scott (1928), Weinberg and Nichailesco (1928). A primary infection of cattle with *B. welchii*, if it does occur, appears to be of but little importance, and hence it is very unlikely that *B. paludis*, which so closely resembles *B. welchii*, is of any importance as a cause of disease in these animals.

Sheep, more than any of the other domestic animals, show a peculiar susceptibility to infection with a variety of the pathogenic anærobic bacteria. Thus, *V. septique* is incriminated in the diseases " bradsot " and " braxy " in Europe, the *B. œdematiens* in the " black disease " of sheep in Australia and New Zealand, *B. chauvœi* as the cause of " black-quarter " of sheep in Europe and America, and the " *lamb dysentery bacillus* " as the cause of disease of lambs in this country. *B. welchii* has been accused as being responsible for disease in sheep in comparatively few instances, and the relative frequency of *B. paludis* and *B. welchii* infections in these animals and the extent to which they occur must remain an open question until the results of carefully conducted investigations become available.

Summary.

From twelve cases of disease in sheep in which the muscular tissue presented lesions characteristic of those produced by the pathogenic sporulating anærobic bacteria, a new species of pathogenic sporulating anærobic bacillus has been isolated. This bacillus has been named *B. paludis*.

In five of the cases *B. paludis* was present in the infected muscular tissue in pure culture, and in all twelve cases smears from the muscular tissue showed the bacillus to be present in very large numbers. *B. paludis* is considered to have been the cause of the disease which brought about the death of the animals.

B. paludis presents many features in common with *B. welchii*. The chief feature distinguishing between the two bacilli is the specificity of their toxins.

Descriptions of the material from which *B. paludis* was isolated, the morphological and cultural characters of the bacillus, its pathogenic action, and its toxin, together with the differentiation of the bacillus from *B. welchii*, have been given in detail.

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STUDIES ON BOVINE MASTITIS.

II. Diagnosis by Means of the Reaction of the Milk to Brom-cresol-purple.

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As long ago as 1889 Storch showed that milk from diseased udders was altered in chemical composition and less acid than normal, and in 1911 Hoyberg and Rullmann reported independently that milk with a high leucocytic count had a low titratable acidity. The first accurate measurements of true acidity were made in 1919 by Baker and van Slyke, who were able to demonstrate correlation with increased cell or bacterial count.

In recent years the reaction of milk has been freely used as a test for mastitis, and increased alkalinity has sometimes been taken as an indication of the presence of bacteria with consequent lowering of the keeping quality of the milk. Clearly, if increased alkalinity does denote an increased number of organisms its value as a measure of keeping quality will depend on the growth characteristics of the bacteria present ; for if of mastitis type, and therefore in most cases one of the types of streptococci, or bacillus pyogenes, they may not multiply in milk under the usual conditions of distribution. Furthermore, if the alkalinity is due to changes determined by the reaction of the body tissues to the presence of streptococci, and not directly to the metabolic products of bacteria growing in the udder, it is unlikely to be an indicator of keeping quality. The alkalinity itself may, on the other hand, be so great as to interfere with rennet coagulation and cheesemaking, so making the detection of the quarters giving alkaline milk the essential.

Therefore, in discussing the diagnostic value of the reaction test it is necessary to consider the changes which are correlated with the alkaline development, if not its cause. There are also certain outside factors which may affect its practical application.

Indicators and Methods of Use.

Brom-cresol-purple (dibromo-ortho-cresol-sulphonpthalein) is a suitable agent for detecting small variations in the hydrogen ion concentration of milk, and for this purpose was first used by Clark and Lubs (1917). Normal milk, *p*H 6·5-6·6, appears a pale dove-grey, whilst as alkalinity increases the colour becomes more and more purple. Brom-thymol-blue (dibromo-thymol-sulphonpthalein) is another eminently suitable agent, and has a definite advantage over brom-cresol-purple in that the change of colour is from greenish yellow at *p*H of normal milk, through yellowish green at about 7·0, to a definite bluish green ; milks which are abnormal in opacity or in other ways are thus more easily read. The superiority of these two

indicators over others which have been used for the purpose, *e.g.*, rosolic acid alcohol, alizarin, and litmus, lies in the fact that colour changes can be appreciated at *p*H values only slightly in excess of that of normal milk, and that the common range of reaction in the alkaline direction can be practicably divided at eight or more points where colours are easily distinguishable from one another. Many grades of increase of hydrogen ion concentration can also be appreciated.

Brom-cresol-purple was used in most of the work to be here described ; the solution contained 0·9 grams per litre, and two or three drops (0·05 c.c. or 0·075 c.c.) were used to each 3 c.c. of milk. Comparisons have shown that one drop (0·025 c.c.) per c.c. milk, *i.e.*, one part of the indicator in 44,444 parts of milk, is the most convenient for reading ; any number of c.c. can thus be dealt with simply. Brom-thymol-blue is best used in a 0·5 per cent. solution in 10 per cent. alcohol, one drop (0·025 c.c.) being added for each c.c. of milk.

Electrometric Determination of pH.

This was made as a check on the colorimetric readings, the quinhydrone electrode as described by Bülmann being used against a standard 3·5 per cent. KCl calomel electrode. Constant potential was obtainable almost immediately, and, as shown by Lester (1924), the lowering of acidity due to loss of carbon dioxide, which occurs with the platinum hydrogen electrode, was thus avoided.

I have to thank Sir Charles Martin for his kind assistance in affording me facilities to carry out these determinations at the Lister Institute.

Standards.

In order that readings may be accurate, a colour standard of known reaction is necessary. This was prepared from " Certified milk " which always came from the same source and was nearly constant in reaction ; the titratable acidity was tested on many occasions, and never exceeded 16 c.c. N/10 NaOH per litre. Three c.c. of milk were pipetted into each of eight tubes and N/10 NaOH added ; the amounts and approximate *p*H values determined electrometrically are given in Table I.

TABLE I.

METHOD OF PREPARING STANDARD SERIES AND APPROXIMATE *p*H VALUES.

No. of Tube.	1	2	3	4	5	6	7	8
N/10 NaOH per c.c.	0·0	0·02	0·04	0·06	0·08	0·1	0·12	0·14
Approx. *p*H	6·60	6·70	6·81	6·93	7·06	7·20	7·37	7·60

Baker and van Slyke (1919) used standards containing only half as much alkali as in Table I, and gave correspondingly lower *p*H values, about 6·55-7·1. Golding (1920), Sheather (1924), and

Procter and Mattick (1925) found that a standard set prepared with proportions of alkali similar to those in Table I was necessary in order to cover the common variations found in diseased milks. Unfortunately, Procter and Mattick quoted the *p*H values given by Baker and van Slyke as applying to their own standards ; but, since they used proportions of alkali which were twice those of Baker and van Slyke, their *p*H values must have been approximately 6·6-7·6. Table I also shows that the hydrogen ion concentration is not a linear function of the proportion of alkali, but rises steeply as the casein becomes saturated.

The actual colours obtained in making a standard by adding NaOH are pinker than those of milk of the same reaction but without NaOH. This difficulty can be evaded by adding one drop of a 1/500 solution of methylene blue to each tube of the standard set.

A permanent standard besides being always ready has the advantage that it renders readings made at intervals over long periods strictly comparable. An endeavour to produce one has unfortunately been unsuccessful. Toluol, chloroform, sodium fluoride, alcohol, thymol, heat, and other agents have been tried, but none has been found which will preserve milk containing brom-cresol-purple without affecting the colour. Tubes of paint, painted wood, and cardboard surfaces have been tested, but all either fail to give a suitable range of colours or fade. Alcoholic thymol, as recommended by Sheather (1924), and alcohol alone were the most satisfactory, but even milk preserved in this way differed from fresh normal milk when compared a week or two later. Consequently, in the work reported a new standard was made up when required from fresh certified milk ; this remained unaltered for many days at 0° C., and was for all intents and purposes satisfactory.

Factors, other than Disease, known to Affect the Results.

Fat content, provided the milk is of the same kind as the standard, the only factor that frequently and seriously interferes with the readings is the percentage of fat. This varies greatly in different cows of the same or different breeds, and also according to the stage of lactation and the period during the milking at which the sample is taken.

In order to measure accurately the influence of fat, eight complete sets of standards were tested, using milks containing 1, 2, 3, 3·5, 4, 5, 6, and 8 per cent. of butter fat. These were made up with separated milk and 32 per cent. cream, both from certified milk.

From the extract of the results, given in Table II, it can be seen that, taking 3 per cent. as a standard, although a variation of 0·5 per cent. makes little difference, a variation of 1 per cent. so alters the colour as to interfere considerably with the accuracy of the test ; whilst one of 2 per cent. makes the test nearly valueless, because a sample might be regarded as normal, doubtful, or abnormal merely according to the percentage of fat. The table also shows that if the samples are allowed to stand overnight the error is to a large degree

removed, and that centrifuging enables accurate results to be obtained in all samples that are not, for some other reason, grossly abnormal.

Apart from milks containing an unusual amount of fat, certain samples are encountered which are entirely unreadable owing to the presence of blood or albuminous material, or because they are deficient in casein and markedly more transparent than normal. Samples of the latter type have usually an absolute alkalinity.

TABLE II.

Effect of Amount of Fat.

Readings	1%	2%	3%	3·5%	4%	5%	6%	8%
At once	7	6	5	4-5	4	3	1-2	1
Overnight	5-6	5	5	5	4-5	4-5	4	3-4
After centrifuging	5	5	5	5	5	5	4-5	4

Each tube contained 3 c.c. milk and 0·24 c.c. N/10 NaOH, *i.e.*, was a standard No. 5 tube. They were read at once, after standing overnight, and after centrifuging, against corresponding standard sets ; the figures represent the tube of the standard series which each matched.

Period of Milking.

Samples have been taken from various cows at intervals throughout the milking, from animals giving milk of normal reaction, and from animals showing different degrees of abnormality. The first milk nearly always gave a higher reading than other portions, and this was correlated with a smaller percentage of cream.

Stage of Lactation.

The secretion from a newly-calved cow cannot be tested satisfactorily, the colour being made distinctly paler and often greyish by the presence of a relatively large amount of albuminous material. During the first few days of lactation it is, nevertheless, recognisably more acid than normal. From about the ninth day onward the milk appears to be normal in its content of albuminous material and in reaction ; and, provided no mammary disturbance supervenes, remains so until the latter part of the lactation period. Then there appears to be a tendency to the development of a less acid reaction.

Storage.

It is naturally assumed that a test for the reaction of milk should be made as soon as possible after removal from the udder, and in earlier work all tests were made at the farm. But, as practical considerations often make it impossible to examine all samples immediately or even after the lapse of a similar number of hours, it was decided to test the effect of storing samples for various periods under various conditions.

Provided samples were obtained with precautions to ensure the absence of gross contamination, a delay of four hours in transport at summer temperatures, followed by storage overnight at 0° C., or in a cool room (temp. 12-15° C.), had no appreciable effect on the reaction. Even at average room temperature change was not rapid, *e.g.*, in April 24 samples of various initial reactions were taken with the usual precautions, and, after transport occupying two hours, were placed at 0° C. for 18 hours, then at 20° C. In none had the reaction undergone appreciable change after five hours at this temperature, and after a further 24 hours only two showed distinct change.

Having shown that brom-cresol-purple had no germicidal effect in four times the proportions used, the effect of continued storage at about 15° C. was tested on over 50 samples of average character containing streptococci or staphylococci, one drop of indicator being added per c.c. In no case was change perceptible in less than 24 hours and in few cases not even in 48 hours.

Viewing the results reported in this section, which have been confirmed throughout the work, it is seen that unless samples are greatly contaminated the reaction does not change rapidly ; and it is only samples containing large numbers of organisms able to multiply at moderate temperature that are likely to change quickly even in summer.

It therefore seems doubtful if the criticism of Sheather's results (1924) by Procter and Mattick (1925) is justified. It is probable that some of his samples had actually become acid between the times of milking and testing, but that was obviously not the case in the samples tested on the farm and found acid. There seems little reason to doubt that the animals concerned were actually giving a milk of abnormal acidity. I have myself seen numerous instances of this, and Bischoff (1930) has reported numerous cases.

Brom-cresol-purple Papers.

These are prepared by soaking ash-free filter paper in a saturated watery solution of the indicator, and were first used by Golding (1921). An alkaline reaction is shown by a purple colour which develops within a minute or two after dipping the paper in the milk to be tested. Much comparative work has been done in this Institute with tubes and papers, because it is the paper form of the test that the farmer himself would apply.

The readings obtained with papers are little affected by the percentage of fat or colour of the milk itself. This is a distinct gain, but the nature of the test agent is such that readings are not easily made with more than a moderate degree of accuracy. At first it was found that a distinct discrepancy was likely to arise between results obtained by means of tubes and those by means of papers, *e.g.*, of 508 samples tested in one series, and read as normal, intermediate, or abnormal, there was definite disagreement in 6·0 per cent., and partial disagreement in 13·1 per cent. ; and even long experience has

not entirely removed the chance of error. Moreover, in a large number of these tests readings were made under chosen conditions and by comparison with standards. How much greater must be the probable error when the papers are read by one who is at least comparatively inexperienced in making these readings, and who has not, and has probably never had, any standard for comparison. Under these conditions only papers which are frankly purple can be considered, and a large proportion of abnormal milks would thus be missed.

The Value of the Reaction in the Diagnosis of Mastitis.

Van Slyke and Baker (1919[2]), found that of the first 308 milk samples examined 83 per cent. had a reaction below 6·76, and this they regarded as the upper limit of the normal reaction, which was usually between *p*H 6·5 and 6·7. Although their work was in the first place directed towards the detection of added water, and acid or alkaline salts, they noticed that milks of a lower acidity than normal frequently showed an excessive number of leucocytes. In a subsequent article by Baker and van Slyke (1919) it was shown that of 570 samples 17 were less acid than normal ; of these, 11 showed over ten million bacteria per c.c. when tested by Breed's method of direct count, and over one million per c.c. when sown into plates.

Baker and Breed (1920), continuing this work, compared the reaction of 124 samples with the number of leucocytes, the number of epithelial cells, and the presence of streptococci. Though certain individual samples diverged widely, the average number of leucocytes increased fairly regularly from 46,000 per c.c. in samples of *p*H 6·5-6·6, to four million at *p*H 6·8-7·0 and 16 million at *p*H values over 7·0. Epithelial cells also increased, though less regularly, from 75,000 to 560,000. The percentage containing streptococci, which were only recorded when four or more were seen, rose gradually from 3 per cent. in milks of normal reaction to 90 per cent. in milks of *p*H 7·0 or over. Nevertheless, no group was entirely free and many samples of high reaction showed no streptococci. The reaction, however, shows a relation to the other criteria that is rather surprising when it is remembered that in this country four separate workers have found it necessary to use a standard series of range 6·6-7·6. Animals apparently normal in other ways frequently show reactions up to 6·9 or 7·0.

Golding (1921) tested samples from 172 quarters of 13 breeds and found that 169 fell into his groups 1, 2, and 3, *i.e.*, *p*H approximately 6·6-6·85 ; these he considered normal. He had no opportunity to seek the cause of the higher alkalinity of the other three.

Sheather (1924) applied the brom-cresol-purple test to 349 samples, using a standard preserved with alcoholic thymol. Examination was also made for bacteria and abnormal deposit, *i.e.*, greater than 0·03 c.c. per 10 c.c. Of 167 samples which appeared normal in other ways, 92 per cent. gave a normal reaction ; of 137 samples giving a normal deposit but showing streptococci (65) or diphtheroids (72), 65 per cent. had a normal reaction ; while of 45 samples giving an abnormal deposit, in nearly all cases accompanied by streptoccoci, only 13 per cent. were of normal reaction.

A rather large number of the samples were on the acid side of the standard used ; this may in part have been due to a high standard or to one deficient in cream, the percentage of samples appearing purple being thereby decreased. Also many samples were sent by veterinarians in the country and were probably in the post for 12-24 hours, and in summer this may have affected the

reaction if the initial content of organisms was considerable. However, precautions against gross contamination were apparently taken, so that it seems unlikely that more than a small number were examples of acidity due to staleness. As suggested earlier, the abnormal acidity in some cases was clearly due to factors operating in the udder itself.

Procter and Mattick (1925), examined 3,473 samples from one herd of about 30 cows, of which 92·8 per cent. fell into their standard colour groups 1-3, 3·9 per cent. corresponded to group 4 or 5 and were therefore considered doubtful, and 3·3 per cent. were considered definitely abnormal as the colour indicated a reaction equal to that of group 6, 7, or 8. The *p*H values were probably about 6·6-6·9, 6·9-7·1, and over 7·1 respectively. No comparative cultural or other examination was made, but the percentage of normal reactions was sufficient to encourage a more detailed investigation.

Gloy and Bischoff (1929) have described in detail experiments that add much to our knowledge of the value of the reaction test in the diagnosis of mastitis. Using " Thybromol " (presumably brom-thymol-blue), they tested the milk from 262 quarters of 69 cows in five herds. The results were compared with those obtained by electrometric determination of hydrogen ion concentration, by estimate of chloride content using Drost's method, and by microscopic and cultural examination for streptococci of the type regarded by Seelemann (1928) as mastitis streptococci. It is not clear that all samples were tested culturally, but presumably any showing microscopic or other indication of possible disturbance were so tested. It is unfortunate that no indication is given of the method used in determining the hydrogen ion concentration electrometrically, because the values given as normal, *p*H 6·3 to 6·5, and which occur most frequently in otherwise normal milks, are distinctly lower than those found by other workers. Further, the *p*H value given for milks which showed in other ways definite evidence of disturbance is decidedly low ; in only a small number of cases greater than 6·8 is it a figure which is generally regarded as about the upper limit of normality. Moreover, it is surprising that the change from yellowish green to greenish yellow is reported as occurring at 6·65, a *p*H value of 6·8 often coinciding with a thymol-blue reading of green and one of about 7·0 with a dark or pronounced green. Although, as the authors themselves say, difficulty was experienced in appreciating any colour change in the lower ranges of *p*H variation, no standard was used, the only comparison being that between quarters of the same cow, the basal tube of which would obviously vary from cow to cow. The effect of this is clearly seen in the lack of any constant relationship between the reaction as determined electrometrically and colorimetrically. Actually a standard can be prepared for this indicator in the same way as for brom-cresol-purple and it shows at least an equally good gradation of colour. Without a standard accurate work is an impossibility.

Early in this article Gloy and Bischoff write that in judging the value of this test it is perhaps not actually necessary that in every case all the affected quarters of a cow should be picked out, but that the detection of a single quarter suffices for the farmer, who can then initiate a thorough examination of all quarters. In summarising their results they have, on these grounds, considered all the quarters of a cow together, and this had led frequently to such a conclusion as that the tests are in agreement when there is only agreement in one or two quarters or between two tests, and sometimes disagreement in other quarters. In most cases, as might be expected, severe cellular disturbances were accompanied by other changes. Nevertheless, as complete details are given, it was possible to make an independent comparison of the results for each quarter and so obtain a more accurate

estimate of the comparative value of the tests used. Gloy and Bischoff seem to have used microscopical and cultural examination as a basis for comparison, but it is not clear what degree of alteration in the microscopic picture was considered definite evidence of abnormality. Five degrees of definite abnormality were recognised, from +, distinct increase of leucocytic types of cell, to ++, and +++, signifying a very great increase.

TABLE III.

Comparison of the Abnormality in Individual Quarters as Measured by Microscopic Examination, Culture, Reaction, and Chloride Content (from Gloy and Bischoff's Results).

pH Electrometric (218 *quarters*)	88 *abnormal.*	130 *normal.*
< 6·3 or > 6·5	75 (85·2%)	55 (42·3%)
6·3 to 6·5	13 (14·8%)	75 (57·7%)
pH Thybromol (262 *quarters*)	106 *abnormal.*	156 *normal.*
Green or very green	29 (27·4%)	5 (3·2%)
Slight or mod. green	27 (25·5%)	14 (9·0%)
Yellow (acid)	3 (2·8%)	0
Normal	47 (44·3%)	137 (87·8%)
Chlorides (257 *quarters*)	102 *abnormal.*	155 *normal.*
> 0·120 gm. per 100 c.c.	37 (36·3%)	15 (9·7%)
0·110 to 0·120 gm. per 100 c.c.	12 (11·8%)	9 (5·8%)
< 0·110 gm. per c.c.	53 (51·9%)	131 (84·5%)

In Table III, ++ or more microscopically, or positive cultural results, are taken as evidence of mastitis, and of 106 samples thus classed as abnormal and tested colorimetrically only 55·7 per cent. were recorded as in any degree abnormal in reaction; of 88 such samples tested electrometrically 85·2 per cent. were abnormal in reaction. Of 156 samples thus classed as normal 12·2 per cent. were abnormal colorimetrically, while of 130 such samples tested electrometrically 42·3 per cent. were abnormal in reaction. Incidentally this reflects the lowness of the standard taken for the latter work. Moreover, if a smaller degree of microscopical alteration be considered as evidence of definite disturbance an analysis similar to that in Table III shows that the percentage of agreement is but little altered. It is also clear that the amount of chlorides present bears no constant relation to the other evidence of disturbance. Gloy and Bischoff conclude that the thybromol test is not to be represented as a universal method but as an accessory, and state that in 75 per cent. of cows examined the test pointed out existent disease; whilst in 16·2 per cent. which were in the early stages it failed. From a comparison of quarters, as in Table III, it is evident that the reaction test, as also the chloride test, had erred in greater degree and in both the positive and negative senses.

Bischoff (1930) reports the occurrence of abnormally low acidity in two herds, one of 300 cows and another of 120. In the latter 55 animals were tested and 41 were found to give a milk of abnormally low *p*H, 6·1-6·3, and abnormally high titratable acidity, over 8·0 to 10·0 degrees on the Soxhlet-Henkel scale. This occurred in young and in old animals, in those newly calved or advanced in lactation, and in those whose udders otherwise were normal or abnormal; it became highest in October and November and decreased at grass, but apart from that the food seemed to have no influence.

At this Institute work on mastitis during the past two years has afforded ample opportunity to study the diagnostic value of the reaction as indicated by brom-cresol-purple. In the first place it may be said that the reaction shows a considerable degree of constancy; *e.g.*, in six otherwise normal quarters the variations of *p*H have not usually been more than 0·2 on successive days, little greater in successive weeks, and the milk has remained normal for many months. Certain infected quarters have on the other hand nearly constantly given an abnormally high reaction, while other quarters may have shown a gradual rise and fall. Observations made on a large number of other animals, both here and on the many farms at which we have worked, agree with these results in showing that a normal or abnormal reaction is usually the expression not of a passing condition but of one that exists over a considerable period. Sudden alterations, which are nearly always in the direction of abnormality, usually coincide with other evidence of disturbance or acute mastitis.

Experimental Results.

Series 1.—In our earliest work the reaction indicated by brom-cresol-purple was compared with any evidence of abnormality that could be ascertained from the history or by clinical examination. On three farms 155 quarter samples were tested once, 91 twice, and 234 on three occasions. The results are given in Table IV.

TABLE IV.

Series I, Evidence of Abnormality Obtained from Complete Clinical Examination Compared with Reaction of the Milk.

From history and clinical examination.	*Reaction normal* (1, 2 *or* 3).	*Intermediate* (4 *or* 5).	*Abnormal* (6, 7 *or* 8).
88·3% normal	73·0%	6·0%	9·3%
3·3% doubtful	1·7% } 77·3%	1·2% } 7·6%	0·4% } 15·1%
8·4% abnormal	2·6%	0·4%	5·4%

Many of the apparently healthy animals which gave milk of an abnormal reaction were shown to be reaching the end of their lactation period, and in these cases, which formed 5·5 per cent. of the total animals tested, the raised reaction was probably physiological; in 1·8 per cent. an intermediate reading is probably ascribable to a similar cause. The figures in the first line of columns 3 and 4 of Table IV would thus be reduced to 3·8 per cent. and 4·2 per cent. respectively.

Thus, disregarding the physiological factor operating in at least 7·3 per cent. of cases, there was complete or partial disagreement with the clinical findings and history in 13·2 per cent. Unfortunately it was not possible to follow up the history of all these cows.

Series 2.

As mastitis, and therefore the characters of the milk, are dependent to a large extent on the presence of streptococci, one important criterion of the diagnostic value of brom-cresol-purple or of any reaction test is the association of an altered reaction with the presence of streptococci of mastitis type (see this journal, 42, 213).

Comparative observations have therefore been made, which in some animals have extended over a period of 18 months, and in certain cases have been continued to the end of one lactation period and through part of the succeeding one. Twenty-four animals were included in this series, and in about half of these the tests have been continued for twelve months or longer. As these tests proceeded it became increasingly clear that as lactation approaches its end the quarter, whether infected or not, tends to give a milk of less acid reaction ; this is most marked, however, in infected quarters, and especially in those that have shown a variable reaction earlier. It seems that in normal quarters the reaction rises simultaneously in all quarters, and is usually associated with a deliberate attempt to end the lactation period. In infected quarters there often occurs a series of rises and falls whose average plane gradually rises until, a long time before the quarter is dry, a nearly constantly high reaction is registered ; this may be in one or two quarters only, the others being quite normal.

During the first six months observations were made at about weekly intervals, these being then gradually increased until at the present time examinations are being made once in two months. Samples were taken so long as it was possible to obtain them. In certain abnormal animals, therefore, they were taken for a considerable period after the quarter had ceased to give milk of normal appearance. The inclusion of results obtained with such material would have entirely obscured the value of the reaction test as an indicator of disturbance unassociated with obvious abnormality ; the inclusion of reactions believed entirely due to the physiological factor of drying would have had a similar effect. Consequently, all such reactions have been separately considered.

TABLE V.

Comparison of the Reaction of Milk from Infected and Healthy Quarters.

Total samples and type of quarter.	*Reaction normal (Standard tubes 1, 2, or 3).*	*Reaction intermediate (standard tubes 4 or 5).*	*Reaction abnormal. (Standard tubes 6, 7 or 8).*
Uninfected : 933 samples ...	844 (90·4%)	66 (7·1%)	23 (2·5%)
Infected : 302 samples ...	175 (58·0%)	30 (9·9%)	97 (32·1%)

In Table V are recorded 933 samples from 50 uninfected quarters and 302 samples from 33 quarters infected with streptococci. In the course of other work on the general question of diagnosis bacterial

counts were being made, and so were available for comparative purposes. In infected quarters little relation could be found between the actual streptococcal counts and abnormal reactions, but long infection did appear to have some significance.

Analysing the results a little further, it was found that out of 50 uninfected quarters 47 gave normal reactions on nearly every occasion ; two were a little variable, usually giving normal or intermediate results ; and one-quarter during the latter two-thirds of these tests always gave an intermediate or abnormal result. Milk from this quarter, however, gave on nearly every occasion plate counts of 1,000 or less staphylococci per c.c. ; this is unusual, and may account for the altered reaction. Of the 33 infected quarters considered in Table V, 14 gave milk that was usually of intermediate reaction, but frequently abnormal ; the remaining 19 quarters, although showing some variability, commonly gave milk of normal reaction. Fourteen other quarters infected with streptococci were, when first tested, giving milk abnormal both in naked eye characters and in reaction, and these characters remained nearly constant.

While this series of observations has been in progress four quarters have become infected. In one case the reaction was affected almost at once ; but in the others, although tests were made in two cases four and eight times in two months respectively, and in one case 14 times in five months, the reaction remained normal until lactation had nearly ceased.

This series thus showed that a single normal reaction is of no practical significance, and that a series of normal results may be given by an infected quarter. On the other hand, a single abnormal reaction, and in greater degree a series of variably or constantly abnormal reactions, may be usefully regarded as an indication that some other abnormality may be present.

Series 3.

It has been shown that in order to obtain accurate results with the tube form of the test it is necessary to centrifuge the samples, much time and materials being thus required. Moreover, it has been made clear that even when reactions are obtained with tubes it is only the samples of definitely decreased acidity that are to be taken as indicative of mastitis, and long experience has practically removed any error in reading such samples by the paper form of the test. In our recent diagnositic work, therefore, only the paper form has been used. The results obtained reflect the best results that could be expected on the farm, where the tube form of the test, although able to give somewhat more accurate results, is impracticable.

In 811 samples, from not less than 300 different quarters, the reaction was thus obtained and compared with the amount of deposit and with cultural examination for streptococci of mastitis type. Four hundred and eighty-five samples were from uninfected quarters, and 326 samples from infected quarters. The results are given in Table VI.

TABLE VI.

COMPARISON BY MEANS OF BROM-CRESOL-PURPLE PAPERS OF THE REACTION OF MILK FROM INFECTED AND UNINFECTED QUARTERS.

Reaction.	*Source of Sample.*		*Amount of Deposit from* 10 *c.c.*	
	Uninfected qr.	*Infected qr.*	$<$ 0·01 *c.c.*	$>$ 0·01 *c.c.*
Normal (566)	449 (79·3%)	117 (20·7%)	508 (89·8%)	58 (10·2%)
Intermediate (159)	26 (16·4%)	133 (83·6%)	64 (40·3%)	95 (59·7%)
Abnormal (86 samples)	10 (11·6%)	76 (88·4%)	18 (20·9%)	68 (79·1%)

It is probable that in most cases the definitely or partially abnormal reaction of milks from quarters free from streptococci, *i.e.*, of 26 plus 10, or 7·5 per cent., of uninfected quarters, was due either to the fact that the end of lactation was approaching or that there was disturbance due to other organisms. On the other hand, 117 samples which were of normal reaction and formed 20·7 per cent. of total normal reactions, contained streptococci and came from quarters which in a large proportion of cases had been infected for many months ; in fact, they formed 35·9 per cent. of this category. These are instances of definite failure of the test, so showing again that a normal reaction cannot be regarded as evidence of freedom from mastitis streptococci.

Nevertheless, it is evident that in uninfected quarters the percentage of reactions is highest in the normal group and lowest in the abnormal ; while in samples from infected quarters it is *vice versa*. Columns 6 and 7 show that the amount of deposit varies in a similar manner. The question of the amount of deposit that is significant and its relation to other factors will, however, be taken up in a subsequent publication on the general question of diagnosis.

THE CAUSE OF THE VARYING REACTION OF MILK.

The various workers who have studied the altered reaction of the milk from diseased udders have expressed widely divergent views regarding the cause of the change. Thus, Storch (1889) and Hoyberg (1911) believed it to be due to the passage of blood serum into the milk, and Bahr (1913) suggested the passage of alkaline phosphates ; Rullmann, on the other hand, sought, though unsuccessfully, to demonstrate bacteria formative of alkali. Since these earlier observations, the changes correlated with the altered reaction have been extensively investigated, but it cannot yet be said that the basic factors are thoroughly understood.

It is at once evident that the alteration of reaction finds its origin in changes located in the particular quarter whose secretion is affected, for, except in animals near the end of lactation, it is very unusual to find the four quarters uniformly high. The alteration may conceivably be due to the presence of the products of bacterial metabolism, the resultant tissue reaction, or actual damage to the lining endothelium of the alveoli, allowing substances, unusual in kind or quantity, to pass in to the milk.

It is unlikely that the alkali is a direct product of bacterial metabolism, as was thought possible by some of the earlier workers, because the organisms usually found in the altered milks, when suitably tested *in vitro*, *e.g.*, in milk, show a capacity to attack lactose, producing not alkali but acid ; and there seems no reason to suppose that they do not utilise the lactose present in the milk of the udder. It is not known, however, to what extent the sugar is split and whether it reaches the lactic acid stage or is even further broken down. But assuming that acid is formed, it is obviously also a probability that the tissues make some effort to neutralise the acid products, and conceivable that the alkaline change is an effect of overneutralisation. Another view would be that the products of bacterial growth so disturb the normal chemical changes as to alter directly the normal CO_2—bicarbonate, phosphate, or other delicately balanced mechanism.

It is generally believed (Heinemann, 1921 ; Hawk, 1925) that the reaction of normal milk is controlled by the relative amounts of acid and basic phosphates, and on that basis the changed reaction of the secretion of abnormal udders may be actually effected in a similar manner. Moreover, the change of reaction can be imitated *in vitro* by adding a suitable amount of alkaline phosphates. Nevertheless, the relative amounts of acid and basic salts, such as phosphates, are directly connected with the carbon dioxide—bicarbonate balance, any alteration in one immediately affecting the other. It is accepted that the reaction of blood is controlled by the comparative amount of CO_2 present in combination with the hæmoglobin or in solution, and in the form of bicarbonates, the latter forming the so-called " alkaline reserve " ; and the reaction of the tissues is believed to be similarly adjusted.

In milk CO_2 is present in variable amount, 10-80 per cent., and van Slyke and Baker (1919[1]) have shown that the acidity of normal milk containing 10-15 per cent. of CO_2 is much less than it would be were the CO_2 uncombined ; supposing, therefore, that part is in the form of bicarbonate, calculations based on the law of mass action and direct experimental evidence have shown that in normal milk the molecular ratio of H_2CO_3 to bicarbonate is as 1 : 2. These authors have also shown that actual removal of CO_2 from a given sample raises the *p*H value ; but that, on the other hand, abnormally high total CO_2, which was determined by treating milk with 20 per cent. lactic acid and absorbing the CO_2 with 5 per cent. NaOH, was frequently associated with low hydrogen ion concentration and low titratable acidity. For example, certain samples showed a CO_2 content of 7-10 per cent., *p*H value 6·5 to 6·65, and titratable acidity 16-20 c.c. N/10 NaOH per cent., while another sample gave CO_2 86 per cent., *p*H 7·16, and titratable acidity 4 c.c. N/10 NaOH per cent., *i.e.*, it appears that the relative amounts of combined and uncombined CO_2 vary widely with such evidence of abnormality as change of reaction.

The view that the reaction is dependent on acid products of metabolism is nevertheless made difficult because the commonly

occurring long infection with small numbers of staphylococci fails to induce the relative alkalinity, although these organisms are also able to use lactose. Furthermore, the fact that the change of reaction appears to be associated usually with some evidence of mastitis, acute or chronic, lends support to an interpretation of a different kind ; *viz.*, that the change is due to actual damage of the alveolar membrane, causing a greater permeability with as a result the passage of a substance such as serum or lymph or, if the damage is less severe, possibly only substances such as the salts of the blood. It is clear that the salts, being of lower molecular weight, would dialyse more easily than serum, which would presumably require a definite trauma. Moreover, in an infected quarter the reaction bears no relation to the bacterial content, but either remains normal or shows a tendency to rise and fall until the average plane is abnormal. Such facts harmonise with a conception of altered permeability due to a damaged alveolar membrane.

Experimental Results.

If the change were due to simple addition of serum or lymph, both fluids with a higher *p*H value, it might be expected that the addition of various amounts of serum or lymph to normal milk containing brom-cresol-purple would produce a series of colours similar to those obtained with the alkaline milk of diseased udders. This has been attempted, but with even 50 per cent. of serum, an amount which distinctly alters the characters of milk, the colour obtained is in no way similar. Further, such an amount of serum or lymph greatly increases the total coagulable proteins, and this is not the case in macroscopically normal, but alkaline milks, or in the great majority of distinctly altered samples, although, according to van Slyke and Baker (1919[2]), the amount of albumin and proteins other than casein are frequently slightly greater than normal. In addition, I have not been able to correlate the degree of alkalinity, as indicated by brom-cresol-purple, with the serum content measured by means of the precipitin test.

Wells and Osborne (1926) showed that lacto-globulin is the only one of the four chemically and immunologically distinct proteins of milk that can sensitise to ox serum, or cause reactions in animals sensitised to ox serum. It is thus the only one that will act as antigen in precipitin tests made with an antiserum prepared again ox serum. The use of a precipitin technique to estimate the likelihood of increased alkalinity being due to an influx of blood serum or serum constituents depends therefore on the assumption that the amount of globulin is always proportional to the other constituents which have passed from blood into milk. If, as has been suggested by certain workers, whole serum passes directly, the assumption would be correct ; in this connection, however, it must be remembered that any results obtained with colostrum may be too high, as in that fluid the proportion of globulin to albumin is relatively increased. Any conclusion regarding

the legitimacy of accepting the lacto-globulin content as a measure of the presence or amount of blood serum must be governed by our knowledge regarding the source of lacto-globulin and other proteins present in normal milk and colostrum. Jones (1926) showed that mastitis milk gave precipitates with a cow serum antiserum in much higher dilutions than normal milk.

Antisera were prepared by immunising rabbits with ox serum, a method described by Daranyi (1928) as satisfactory, being used. Rabbits were given a dose of serum subcutaneously at 9 a.m., followed by an intravenous dose at 4 p.m., the amount of the subcutaneous injection being increased from 1 c.c. to 10 c.c. in subsequent injections, and the intravenous decreased from 10 to 1 c.c. One rabbit was lost at the second and one at the third injection, and the serum obtained from the other four after five injections, although useful (titre 1/10,000-1/20,000), was not as high as reported by Daranyi, or as can be obtained according to Dean and Webb (1926) by a long and interrupted series of subcutaneous injections.

Preliminary tests were carried out at room temperature by both the disc methods and after shaking, 0·1 c.c. of antiserum and 0·1 c.c. of a suitable dilution of cow serum giving satisfactory results ; but in testing for the presence of serum in milk it was found preferable to mix 0·1 c.c. of antiserum with 1 c.c. of milk dilution and incubate at 37° C. for two hours, or at 55° C. for a shorter period, the reactions being read by the speed or appearance of flocculation, its character

TABLE VII.

Comparison of Reaction, Deposit, and Serum Pricipitin Titre.

Source of Sample.	*Re-action*	*De-posit.*	$\frac{1}{100}$	$\frac{1}{200}$	$\frac{1}{300}$	$\frac{1}{400}$	$\frac{1}{500}$	$\frac{1}{800}$	$\frac{1}{1000}$	$\frac{1}{2000}$	$\frac{1}{5000}$
Milk + 20% serum	grey	tr.	—	—	—	—	3	—	2	1	$\frac{1}{2}$
Milk + 4% serum	?1	tr.	$2\frac{1}{2}$	2	—	$1\frac{1}{2}$	1	—	tr.	—	—
Milk + 2% serum	1	tr.	2	—	1	—	—	—	0	—	—
Milk alone	1	tr.	1	$\frac{1}{2}$	—	—	tr.	—	0	—	—
Cow 1, colostrum, 1st day	<1	—	—	—	—	3	—	3	—	1	tr.
Cow 1, Noline, colostrum, 3rd day	2	—	—	1	$\frac{1}{2}$	—	tr.	—	—	—	—
Cow 2, Mistletoe, colostrum, 2nd day	<1	—	—	3	—	—	1	—	tr.	0	—
Cow 852 L.H.	2	tr.	2	2	—	—	$\frac{1}{2}$	—	0	—	—
,, 852 R.F.	1	tr.	1	$\frac{1}{2}$	—	—	0	—	0	—	—
,, 017 ? quarter	8	tr.	2	1	—	—	1	—	tr.	—	—
,, B116 L.F.	8	large	3	3	—	—	2	—	1	$\frac{1}{2}$	—
,, B116 R.F.	8	0·03 c.c.	3	2	—	—	2	—	1	$\frac{1}{2}$	—
,, 905 R.F.	7	tr.	1	—	—	$\frac{1}{2}$	—	tr.	—	—	—
,, B101 R.F.	7	0·01 c.c.	2	—	—	1	—	$\frac{1}{2}$	—	—	—

The figures in column 2 are colorimetric measurements of reaction ; the figure 1 = *p*H 6·5-6·6, figure 8 = about *p*H 7·6. In column 3 are given the amounts of deposit from centrifuging 10 c.c. of milk. The figures in the other columns indicate the comparative amount of precipitate obtained at various dilutions of milk ; 3 = +++, $\frac{1}{2}$ = ±, 0 = no precipitate.

and extent, and checked after refrigeration overnight. With this technique it was found that the antisera all gave a satisfactory result (+ or ++) with cow serum diluted 1/10,000, while in higher dilutions the presence of cow serum could still be detected.

Normal milk containing various percentages of added cow serum, and milk alone were first tested, 1 c.c. of dilutions from 1/100-1/1,000 being mixed with 0·1 c.c. of antiserum. From Table VII it will be seen that the normal milk when diluted 1/100 gave a one + reaction, whereas the milks containing added serum all gave a similar reaction in higher dilutions, and that containing 20 per cent. added serum gave a one + at 1/2,000. It was found that normal mixed milk generally reacted in this way at 1/50-1/100, sometimes at 1/150 or 1/200. The degree of reaction, +, given by mixed milk diluted 100 times, is thus approximately the same as that given by serum diluted 10,000-20,000 times, *i.e.*, milk reacts as a ½-1 per cent. dilution of serum. Controls of milk and saline, milk and normal serum, and antiserum and saline were entirely negative, nothing more being observable than a slight cloudiness in the lowest dilutions, and, after standing overnight, a trace of powdery deposit. Milks containing from 1-10 per cent. and 20 per cent. of added serum were used as controls, and early in the work it was noticed that a control milk containing 10 per cent. reacted one + at 1/1,000, whilst a milk with 4 per cent. added serum gave one + at 1/500 ; in subsequent work, therefore, tests were incubated until ++ or +++ reactions were evident in the earlier tubes of the control series used and a one + in the tube whose dilution was 100 times the total percentage of serum. About 90 samples of udder secretion of various reactions, obtained colorimetrically and read after well centrifuging, were tested together with controls of normal milk containing a known percentage of added serum. Table VII contains examples of the reactions obtained with milks of various reactions, normal and abnormal in appearance and deposits.

It was thus found that a normal reaction was seldom correlated with a high precipitin titre, and distinctly alkaline milk generally reacted at a higher dilution than normal milk. A general relationship with the amount of deposit obtained by centrifuging was also evident. On the other hand, there was in some samples a definite discrepancy between the alteration in the milk as measured by the serum-precipitin titre and the reaction. Moreover, colostrum obtained immediately after calving reacted similarly to a 20 per cent. dilution of blood serum in milk, the precipitin titre decreasing to about 1/500 during the next 24 hours, and then more gradually, until about a week later it was similar to that of normal milk. In no case was the reaction of colostral samples more alkaline than normal.

Thus the reaction, deposit, and serum precipitin titre are probably not directly connected, but the coincident expression of separate factors.

Summary.

1. Brom-cresol-purple was used to indicate the reaction of most of the milk samples examined. Although, as found by others, it is a suitable agent for this purpose, accurate readings are more easily made with brom-thymol-blue.

2. The standards of graded alkalinity used by other workers are compared with each other and with electrometric determinations.

3. Factors other than disease which may affect the results are considered.

The amount of fat has a marked effect, the tint of the indicator being masked by a high percentage ; this effect is removed by centrifuging.

Storage for short periods does not vitiate the results. Provided milk was carefully drawn, it was found that the reaction was not seriously altered by storage for three or more days at 0° C. or for 24 hours at 15-20° C. It was only samples containing a large number of organisms able to multiply at moderate temperatures that were seriously changed by storage at 15° C. for 48 hours.

As the end of lactation approaches, the reaction tends to rise in all quarters. This is most marked in animals infected with known disease-producing organisms such as streptococci, but also occurs in normal animals, and is then usually simultaneous in all quarters.

4. In three series of 480, 1,246, and 811 samples respectively, it is shown that a single normal result or even a series of normal results is of no value in the diagnosis of mastitis, because a quarter which is constantly excreting streptococci, or which may have recently shown clinical symptoms, frequently gives a series of normal reactions. On the other hand, a single abnormal reaction, or in greater degree a series of abnormal or doubtful reactions, points to the probability of disease.

In the third of these series the reaction was obtained by means of brom-cresol papers.

5. The findings of other workers relative to the question of diagnosis are analysed.

6. The cause of the decreased acidity or actual alkalinity is discussed. No evidence has been found to support the view that the abnormal reaction is due to an influx of blood serum ; the reaction of the milk shows no constant relation to its antigenic titre in serum-precipitin tests.

"I wish to express my indebtedness to Dr. F. C. Minett, Director of the Institute, for his advice and assistance, and also to acknowledge the help received from Mr. S. J. Edwards."

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IMMUNISATION OF FOWLS AGAINST FOWL POX BY MEANS OF PIGEON POX VIRUS.

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Fowl Pox is a widespread contagious disease which is the cause of heavy annual loss to the poultry industry throughout the world. For many years efforts have been directed to finding a safe and reliable method of immunisation, but until quite recently the results have been unsatisfactory.

Vaccination with heated virus has been practised in America with variable results. This method was advocated by Beach, who prepared a vaccine by heating a 1 per cent. saline suspension of finely powdered crusts for one hour at 55° C. Attempts to standardise the vaccine were not successful. Beach says, in connection with the efficiency of this method: "Vaccine prepared from desiccated chicken-pox virus, while not conferring in the majority of cases complete protection against severe artificial infection, does confer a considerable degree of resistance."

Boener and Stubbs, and Ward and Gallagher, have tried this vaccine but apparently obtained little success.

In assessing the prophylactic value of heated virus it is essential to distinguish between virus which has been killed and that which has only been modified. It is possible that the discordant results reported may be due to this factor. The majority of investigators are agreed that dead virus, whether killed by physical or chemical means, fails to confer immunity. In our experience, virus modified by heat gives inconstant results. Sometimes it has protected against both artificial and natural infection, but at other times it has caused either generalisation of infection in many of the inoculated fowls or has failed to protect.

During the last few years live virus has been extensively employed in America for the protection of fowls against the disease.

While circumstances may sometimes justify the use of live vaccine, such as on heavily infected premises, it is clear from published reports that its employment is followed by severe constitutional disturbances in a considerable percentage of the inoculated fowls. There is usually loss of condition, and frequently there is more or less severe generalisation of infection, while occasionally heavy mortality is encountered. A further objection is that the pox crusts from the inoculated area on the skin increase the existing contamination of the ground, perpetuate the infection, and necessitate the annual inoculation of young stock.

We have frequently observed that the subcutaneous inoculation of live virus, even in minute quantities, may be followed by progressive emaciation. Similar occurrences are met with in the course of natural outbreaks; certain birds, although showing none of the

typical lesions of the disease, gradually become emaciated, and usually die.

The immunisation of fowls by means of live fowl pox virus, whether in weak dilutions or modified by heat, is unreliable and dangerous, and its employment is only justified in exceptional circumstances.

To be of practical value a vaccine must be free from danger, must confer a reasonable degree of protection, and must not introduce infection into a flock.

Doyle and Minett (1927) referred to the marked resistance of pigeons to infection with fowl pox virus, and to the susceptibility of fowls to pigeon pox virus. They showed that although the viruses of fowl pox and pigeon pox are immunologically indistinguishable they differ somewhat in their species adaptation, and that pigeon pox virus protected fowls against fowl pox virus.

The observation that pigeon pox virus protected fowls against subsequent infection with fowl pox virus opened up a new and promising line of inquiry into the problem of immunisation.

Since the publication of that report, further investigation has shown that unmodified pigeon pox virus provides a ready-made vaccine against natural infection with fowl pox.

In 1928, Zwick, Seifried, and Schaaf reported the successful employment of pigeon pox virus for the protection of fowls against fowl pox. They found that the inoculation of fowls with pigeon pox virus was not followed either by mortality or by generalisation of infection ; and that three weeks after inoculation the birds showed considerable resistance to artificial infection and an absolute immunity to natural infection with fowl pox virus.

Lahaye (1928) published similar results to those of the German workers. He claimed, however, that fowls inoculated with pigeon pox virus were completely protected against artificial infection and that immunity lasted for more than one year.

The object of the experiments here described was to assess the value of pigeon pox virus for the protection of fowls against fowl pox under laboratory conditions.

Every experiment was repeated at least three times, and on each occasion the strain of fowl pox virus employed for testing was obtained from a different source ; as similar results were always obtained, it will suffice to give the details of one experiment of each series. One strain of fowl pox virus was particularly virulent, and the results tabulated are those obtained in testing against that strain.

Pigeon Pox Vaccine.

This is prepared by inoculating the plucked breasts of pigeons with pigeon pox virus and, depending on the amount of growth, collecting the crusts between the ninth and twelfth day. The crusts are dried in a desiccator and ground into powder.

The vaccine consists of a 1 per cent. suspension of the powdered virus in 80 per cent. glycerin in 0·85 per cent. salt solution.

Method of Inoculation.

Inoculations were made on the comb for some years, but the method now employed is that introduced by De Blieck and Van Heelsbergen.

The site of inoculation is the front of the thigh, a couple of inches above the tibio-tarsal joint. About twelve to fifteen feathers are removed, and the vaccine is applied lightly to the feather follicles with a small scarifier or a stiff brush. Swelling of the follicles is noticeable from the fourth day, increases up to about the eighth day, and then gradually subsides. (See Fig. 2).

Fig. 1.
Showing Pigeon Pox Nodules on the Pigeon's Breast.

Pathogenicity of Pigeon Pox Vaccine for Fowls.

In order to ascertain the pathogenicity or pigeon pox vaccine for fowls the following experiment was carried out :—

Thirty-two fowls were divided into four groups of eight and each group was placed in a cage. Six birds of each group were inoculated and two were left as controls. The four groups were infected by different routes, *i.e.*, intravenously, intramuscularly, subcutaneously, and *per os*.

Three fowls of each group were given 1 c.c., and three were given 2 c.c. of vaccine, and they were kept under observation for thirty days ; during the period of observation all the birds remained in good condition, and none of them showed lesions.

In view of the importance of determining accurately the infectivity of the vaccine before employing it on a large scale under field conditions, fowls have been kept in contact with those under experiment throughout the investigation.

As some hundreds of fowls have had pigeon pox lesions on the leg, comb, or mouth, there has been every opportunity for infection of the control fowls, but this has never occurred. Out of a total of over a thousand fowls inoculated with pigeon pox vaccine, only six died, and none of them showed pox lesions at the time of death. But as somewhat similar instances follow the inoculation of fowl pox virus, and occur also in natural outbreaks, it is only right, in the light of present knowledge, to attribute these deaths to the action of the vaccine. Such cases are, however, so infrequent as to have little practical importance.

FIG. 2.

Right Leg, showing Swelling of the Feather Follicles after Inoculation with Pigeon Pox Vaccine.

Left Leg, Control.

Test of the Immunity of Vaccinated Fowls against Natural Infection with Fowl Pox.

In the early work undertaken to assess the protective value of pigeon pox vaccine the fowls under test were kept in modern poultry houses with concrete floors. In the first experiment 120 fowls, divided into three equal groups, ranging in age from three to twelve months, were employed. The fowls of one group were inoculated on the leg with pigeon pox vaccine, those of a second group were inoculated on the comb and mouth with fowl pox virus, while the fowls in the third group were left as controls.

In order to allow sufficient time for immunity to develop, the three groups were kept separate until the 21st day after the birds

TABLE I.

SHOWING THE RESULTS OBTAINED FROM TESTING FOWLS INOCULATED WITH PIGEON POX VACCINE AGAINST NATURAL INFECTION WITH FOWL POX.

Group	*Number of Bird*	*Inoculation Material*	*Interval after Inoculation when placed-in-contact*	*Results*			
				7th day	*14th day*	*21st day*	*28th day*
1	1740	Pigeon Virus	21st day	+ m.	+ m.	—	—
	1748	,, ,,	,, ,,	+ m.	—	—	—
	1893	Fowl Virus	7th day	Heavily infected on comb and mouth			
	1912	,, ,,	,,	,, ,,	,, ,,	,,	
	1969	,, ,,	,,	,, ,,	,, ,,	,,	
	37	Controls	—	+ m.c.	Died	—	—
	25	,,	—	—	—	—	—
2	1709	Pigeon Virus	21st day	+ m.	+ m.	—	—
	1710	,, ,,	,, ,,	+ m.	—	—	—
	1904	Fowl Virus	7th day	Heavily infected on comb and mouth			
	1910	,, ,,	,,	,, ,,	,, ,,	,,	
	1922	,, ,,	,,	,, ,,	,, ,,	,,	
	31	Controls	—	++ c.	+++ f.c.	++ f.c.	++ f.c.
	33	,,	—	++ c.m.	++ c.m.	Died	
3	1714	Pigeon Virus	21st day	—	—	—	—
	1738	Pigeon Virus	,, ,,	++ m	—	—	—
	1915	Fowl Virus	7th day	Heavily infected on comb and mouth			
	1965	,, ,,	,,	,, ,,	,, ,,	,,	
	1975	,, ,,	,,	,, ,,	,, ,,	,,	
	23	Control	—	++ c.	++ c.f.	Died	
	24	,,	—	+ c.	++ c.e.f.	+++ c.e.f.	Died
4	1712	Pigeon Virus	21st day	+ m.	—	—	—
	1752	,, ,,	,, ,,	—	—	—	—
	1900	Fowl Virus	7th day	Heavily infected on comb and mouth			
	1977	,, ,,	,,	,, ,,	,, ,,	,,	
	1931	,, ,,	,,	,, ,,	,, ,,	,,	
	21	Controls	—	++ m.	++ m.	Died	
	34	,,	—	++ m.	+++ m.f.c.	++ m.f.c.	+

5	1703	Pigeon Virus	21st day	—	—	—	—
	1744	,, ,,	,,	++ m.	—	—	
	1901	Fowl Virus	7th day	Heavily infected on comb and		mouth	Died
	1943	,, ,,	,,	,, ,,	,, ,,	,,	
	1972	,, ,,	,,	,, ,,	,, ,,	,,	
	20	Controls	—	+ m.e.c.	Died		
	4	,,	—	+ m.	++ m.c.	+ m.c.	—
6	1715	Pigeon Virus	21st day	—	—	—	—
	1717	,, ,,	,,	—	—	—	—
	1907	Fowl Virus	7th day	Heavily infected on comb and		mouth	
	1916	,, ,,	,,	,, ,,	,, ,,	,,	
	1976	,, ,,	,,	,, ,,	,, ,,	,,	
	1979	Controls	—	++ m.	Died		
	1999	,,	—	—	++ c.f.	++ c.f.	Died
7	1706	Pigeon Virus	21st day	+ m.	—	—	—
	1700	,, ,,	,,	—	—	—	—
	1887	Fowl Virus	7th day	Heavily infected on comb and		mouth	
	1895	,, ,,	,,	,, ,,	,, ,,	,,	
	1903	,, ,,	,,	,, ,,	,, ,,	,,	
	39	Controls	—	+ c.m.	++ c.f.	+++ c.f.	+++ c.f.
	32	,,	—	+ c.	++ c.m.	++ c.m.	+ c.
8	1716	Pigeon Virus	21st day	++ m.	+ m.	—	—
	1749	,, ,,	,,	+ c.	—	—	—
	1884	Fowl Virus	7th day	Heavily infected on comb and		mouth	
	1929	,, ,,	,,	,, ,,	,, ,,	,,	
	1941	,, ,,	,,	,, —	,, —	,, —	
	42	Controls	—	++ m.	++ m.c.	Died	—
	46	,,	—				

\+ = One small lesion ; ++ = several lesions ; +++ = numerous lesions.
C = Comb ; m = mouth ; f = face ; e = eye.

had been inoculated with the pigeon pox vaccine. It is unnecessary to give the results obtained in this experiment as they are of little value. None of the birds inoculated with pigeon pox became infected, and only a few of the controls contracted the disease, although most of the birds inoculated with fowl pox virus died.

In a second experiment planned on similar lines, 100 pullets were used, and the results were also inconclusive.

It was evident from these results that, in order to test the vaccine thoroughly it would be necessary to confine the birds in a smaller space, in order to increase the opportunities for infection, and to render the conditions under which they were kept less hygienic than those obtaining in a poultry house of modern design. To attain these ends, most of the experiments were carried out in small galvanised iron cages measuring 2 ft. by 2 ft. by 2 ft. As each cage contained about seven fowls, consisting of vaccinated birds under test, healthy controls, and fowl pox infected birds, it was in itself a complete experiment. The cages were not cleaned out during the test, so that the conditions were less hygienic and the infection tests were more severe than those likely to be met with under natural conditions.

Table I shows the results obtained in testing vaccinated fowls against natural infection.

It will be seen that six out of the 16 vaccinated fowls resisted natural infection ; nine developed slight lesions in the mouth, and one showed a pock on the comb, but all cleared up rapidly without showing any constitutional disturbance or loss of condition. One vaccinated fowl died suddenly on the 28th day, and a post-mortem examination failed to reveal the cause of death.

Out of the 16 controls, 14 became infected, the disease became generalised in ten, and nine of them died. Two of the controls resisted infection.

These experiments prove that pigeon pox vaccine confers strong protection against severe natural infection with fowl pox.

Pigeon Pox Vaccine confers Immunity against Natural Infection with both the Comb and the Mouth Forms of Fowl Pox.

In previous experiments, in order to produce gross infection, the fowl pox virus birds were inoculated both on the comb and in the mouth. In this experiment the fowls were divided into two groups, one group being infected on the comb and the other in the mouth.

Forty-eight fowls were divided into two groups, and each group was housed in a small iron isolation pig-sty.

In each group eight fowls were inoculated on the leg with pigeon pox vaccine, eight were inoculated with fowl pox virus, and eight were left as controls.

The results are shown in Tables II and III.

It will be seen from Tables II and III that 11 out of the 16 vaccinated birds resisted natural infection, four developed mild lesions which cleared up quickly, and one died. Among the control fowls infection became generalised in 11, four died and one resisted infection. The experiment was discontinued on the 28th day, but had it been prolonged over a longer period most of the control birds would undoubtedly have died.

To Determine the Interval required for the Development of Immunity after Inoculation with Pigeon Pox Vaccine.

In this experiment two groups of fowls were inoculated, one with pigeon pox vaccine and the other with fowl pox virus.

Twelve fowls were inoculated on the leg with pigeon pox vaccine and were placed in pairs in cages.

Eighteen fowls were inoculated on the comb and in the mouth with fowl pox virus ; these were inoculated in groups of three, every alternate day, until six groups had been dealt with.

On the tenth day after the fowls had been inoculated with pigeon pox vaccine one pair was put in a cage with the first group inoculated with fowl pox virus, together with two controls. Every other day a pair of pigeon pox inoculated fowls were placed in contact with a

TABLE II.

TEST OF IMMUNITY OF VACCINATED FOWLS AGAINST NATURAL INFECTION WITH THE COMB FORM OF FOWL POX.

No. of Fowl	*Inoculated with*	*Results*			
		7th day	*14th day*	*21st day*	*28th day*
52	Pigeon Pox vaccine	—	—	—	—
69	Pigeon Pox Vaccine	—	—	—	—
1918	Pigeon Pox vaccine	—	+ f.	+ c.	—
64	Pigeon Pox vaccine	Died	—	—	—
70	Pigeon Pox vaccine	—	—	—	—
61	Pigeon Pox vaccine	+ m.	—	—	—
56	Pigeon Pox vaccine	—	—	—	—
73	Pigeon Pox vaccine	—	—	—	—
100	Healthy Control	—	+ f.	+++ f.c.m.	Died
1905	Healthy Control	—	—	+++ f.c.m.	++ f.c.m.
1953	Healthy Control	—	+ c.m.	+++ c.m.f.	Died
104	Healthy Control	—	+ f.c.m.	+++ f.c.m.	++ f.c.
1938	Healthy Control	—	—	—	—
1811	Healthy Control	—	—	++ f.c.m.	++ f.c.
90	Healthy Control	—	+ f.	+++ f.c.m.	+++ f.c.m.
75	Healthy Control	++ m.	++ m.	+++ m.f.c.	Died
192	Fowl Pox virus	++ c.	++ c.	+ c.	—
172	Fowl Pox virus	+ c.	Died	—	—
162	Fowl Pox virus	++ c.	++ c.	++ c.m.	++ c.m.
182	Fowl Pox virus	++c.m.	Died	—	—
200	Fowl Pox virus	++ c.	+++ c.m.	Died	—
187	Fowl Pox virus	+ c.	++ c.	++ c.	+ c.
159	Fowl Pox virus	+ c.m.	Died	—	—
170	Fowl Pox virus	++ c.	Died	—	—

c = comb ; m = mouth ; f = face.
+ = One small lesion ; ++ = several lesions ; +++ = numerous lesions.

TABLE III.

TEST OF IMMUNITY OF VACCINATED FOWLS AGAINST NATURAL INFECTION WITH THE MOUTH FORM OF FOWL POX.

No. of Fowl	*Inoculated with*	*Results*			
		7th day	*14th day*	*21st day*	*28th day*
65	Pigeon Pox vaccine	—	—	—	—
58	Pigeon Pox vaccine	—	—	—	—
53	Pigeon Pox vaccine	—	—	—	—
50	Pigeon Pox vaccine	—	—	—	—
1923	Pigeon Pox vaccine	—	+ m.	—	—
63	Pigeon Pox vaccine	—	++ m.	—	—
55	Pigeon Pox vaccine	—	—	—	—
74	Pigeon Pox vaccine	—	—	—	—
97	Healthy Control	—	—	++ m.f.	+++ f.c.m.
103	Healthy Control	—	—	++ f.	+++ m.f.c.
98	Healthy Control	—	+ m.	+ c.m.	++ c.f.
105	Healthy Control	—	+ c.	++ c.f.	+++ c.f.
93	Healthy Control	—	+ c.	++ c.	+++ c.f.m.
101	Healthy Control	—	—	+ c.	+ c.
59	Healthy Control	+ m.	Died	—	—
86	Healthy Control	—	—	++ c.	+++ c.f.m.
185	Fowl Pox virus	++ m.	++ m.	+ m.	—
164	Fowl Pox virus	++ m.	+++ m.f.c.	Died	—
1792	Fowl Pox virus	++ m.	Died	—	—
188	Fowl Pox virus	++ m.	Died	—	—
198	Fowl Pox virus	++ m.	Died	—	—
1780	Fowl Pox virus	++ m.	+++ m.	+++ m.	++ m.
178	Fowl Pox virus	+++ m.	Died	—	—
161	Fowl Pox virus	++ m.	Died	—	—

c = comb ; m = mouth ; f = face.
+ = one lesion ; ++ = several lesions ; +++ = many lesions.

group infected with fowl pox, and two controls. By this arrangement the immunity of the fowls inoculated with pigeon pox vaccine was tested on the 10th, 12th, 14th, 16th, 18th, and 20th day after inoculation by contact with birds heavily infected with fowl pox.

In each test the birds infected with fowl pox were put in contact on the seventh day, that is, when the lesions were well developed.

The results of this experiment are shown in Table IV.

It will be seen from Table IV that as early as the tenth day after inoculation the vaccine appears to have conferred a considerable amount of protection against natural infection with fowl pox. From the 14th day the immunity was complete. Indeed, the results obtained in this experiment were even more favourable to the vaccine than those of the immunity experiments shown in Table I.

Test of Immunity of Vaccinated Fowls against Artificial Infection.

Experiment 1.—In this experiment pigeon pox virus was passaged by comb inoculation through a series of nine fowls at ten day intervals.

A control fowl was placed in contact with each inoculated bird. As a rule, during the first few passages on the fowl's comb, pigeon pox virus produces somewhat scanty lesions, but as it becomes adapted there is a gradual

increase in growth, and from about the fourth passage good reactions are usually obtained. The period of incubation in the fowl is a little longer than for fowl-pox.

The fowls used for the first four passages, with the in-contact-controls, were re-inoculated with pigeon pox virus on the twenty-first day. The re-inoculated fowls resisted infection, while the controls gave good reactions.

Twenty-one days later these fowls, with those of the fifth to the ninth passages, were inoculated on the comb with fowl pox virus. It will be seen from Table V that the majority of the fowls which were inoculated twice with pigeon pox virus reacted more severely than those which had been inoculated once only, or than the controls.

The important point, however, was that fowls inoculated on the comb with pigeon pox virus were not sufficiently protected to resist a severe artificial infection with fowl pox virus at a subsequent date.

Although many of the fowls which had recovered from pigeon pox developed lesions on the comb when subsequently tested with fowl pox virus, infection did not become generalised in any instance, whereas this occurred in three of the control fowls.

Furthermore, the lesions present on the vaccinated fowls cleared up quickly and their general health was not affected, whereas in non-vaccinated fowls if the disease is allowed to run its course generalisation occurs in many of the birds and there is usually a heavy mortality.

Experiment II.—It was clear from the results obtained in the previous experiment that pigeon pox vaccine did not protect fowls against a heavy artificial infection with fowl pox virus.

In order to ascertain approximately the degree of protection conferred against artificial infection, the following experiment was carried out :—

Thirty-two fowls, sixteen of which had been vaccinated twenty-one days previously on the leg with pigeon pox vaccine, and sixteen healthy controls, were inoculated with varying dilutions of a suspension of fowl pox virus and placed in separate cages.

Eight of the vaccinated fowls and eight controls were inoculated on the comb, and eight vaccinated fowls and eight controls were inoculated in the mouth. The birds were examined on the seventh, fourteenth, twenty-first, and twenty-eighth day. The results are shown in Table VI.

When examined on the seventh day the reactions of the vaccinated birds were, on the whole, slightly more marked than those in the controls. By the fourteenth day a marked change had taken place. In some of the vaccinated fowls the lesions had already disappeared and they were rapidly clearing up in most of the others, while in the control fowls infection was clearly advancing and generalisation had occurred in three cases. Generalisation was evidenced by the appearance of lesions in the mouth following inoculation of virus on the comb, or *vice versa*. By the twenty-eighth day the lesions had disappeared from fourteen out of the sixteen vaccinated birds and generalisation had only occurred in one bird. Among the non-vaccinated birds generalisation occurred in twelve out of the sixteen, and when the experiment was discontinued most of the birds were emaciated and many of them were moribund.

These results were confirmed in a second experiment, in which a strain of fowl pox virus obtained from a different source was used.

It is clear from the results obtained in these experiments that pigeon pox vaccine does not give complete immunity against severe artificial infection with fowl pox virus. Its protective power is, however, sufficient

TABLE IV.

SHOWING RESULTS OBTAINED FROM TESTING FOWLS, AT VARYING INTERVALS AFTER INOCULATION WITH PIGEON POX VACCINE, AGAINST NATURAL INFECTION WITH FOWL POX.

Group	*Number of Birds*	*Inoculation Material*	*Interval after Inoculation when placed-in-contact*	*Results*			
				7th day	*14th day*	*21st day*	*28th day*
1	1733	Pigeon Virus	10th day	+ m.	—	—	—
	1735	,, ,,	,,	+ m.	—	—	—
	1611	Fowl Virus	7th day	Heavily infected on comb and mouth			
	1619	,, ,,	,,	,, ,,	,, ,,	,,	
	1625	,, ,,	,,	,, ,,	,, ,,	,,	
	1779	Controls	—	+ m.	+ m.	—	—
	1812	,,	—	—	—	—	—
2	1747	Pigeon Virus	12th day	—	+ m.	—	—
	1753	,, ,,	,,	—	+ m.	—	—
	1769	Fowl Virus	7th day	Heavily infected on comb and mouth			
	1772	,, ,,	,,	,, ,,	,, ,,	,,	
	1773	,, ,,	,,	,, ,,	,, ,,	,,	
	1783	Controls	—				
	1839	,,	—				

3	1730	Pigeon Virus	14th day	—	—	—	—
	1751			—	—	—	—
	1854	Fowl " Virus	7th " day	Heavily infected on comb and mouth			
	1862			" "	" "	"	
	1928	Controls "	"	+ m.f.	++ m.f.	++ f.	+ f.
	1930			++ c.m.	Died		
4	1707	Pigeon Virus	16th day	—	—	—	—
	1731			—	—	—	—
	1813	Fowl " Virus	7th " day	Heavily infected on comb and mouth			
	1817	" "	"	" "	" "	"	
	1829			" "	" "	"	
	1906	Controls "	"	++ m.	++ m.f.	Died	—
	1908	"	—	++ m.	+++ m.f.	++ m.f.	++ m.f.
5	1729	Pigeon Virus	18th day	—	Died		
	1734			—	—	—	—
	1778	Fowl " Virus	7th " day	Heavily infected on comb and mouth			
	1797	" "	"	" "	" "	"	
	1801			" "	" "	"	
	1932	Controls "	"	—	+ f.	++ f.	Died
	1957	"	—	—	++ m.f.	Died	
6	1711	Pigeon Virus	20th day	—	—	—	—
	1736			—	—	—	—
	1902	Fowl " Virus	7th " day	Heavily infected on comb and mouth			
	1981	" "	"	" "	" "	"	
	1982			" "	" "	"	
	8	Controls "	"	++ f.	+++ f.m.c.	Died	
	12	"	—	—	—	—	—

to prevent generalisation of infection, and eventually to overcome the inoculated virus while the birds remain in good condition.

TABLE V.

Test of Immunity of Vaccinated Fowls against Artificial Infection with Fowl Pox.

Passage.	*Fowl Inoculated on Comb with Pigeon Pox Virus and Control Fowl in same cage.*	*Results.*	*Passage Fowl Re-inoculated and Control Fowl Inoculated on Comb, with Pigeon Pox Virus. Results.*	*Both Fowls Inoculated on Comb with Fowl Pox Virus. Results.*
1	Inoculated	++	—	++
	Control	—	++	++
2	Inoculated	++	—	+++
	Control	—	+	+
3	Inoculated	++	—	++
	Control	—	++	++
4	Inoculated	+++	—	++
	Control	—	++	+
5	Inoculated	+++		++
	Control	—		+
6	Inoculated	+++		+++
	Control	—		+
7	Inoculated	+++		—
	Control	—	Not inoculated	+++
8	Inoculated	+++		—
	Control	—		+++
9	Inoculated	+++		+
	Control	—		—

To Determine whether the Virulence of Pigeon Pox Virus can be exalted by Passage through Fowls.

Pigeon pox virus was " passaged " by comb inoculation through a series of fowls at ten-day intervals.

In addition, a sample of virus obtained from each passage was suspended in 0·85 per cent. salt solution and 1 c.c. was inoculated intravenously into each of two fowls. Control fowls were placed in contact with those inoculated.

The fowls inoculated intravenously with material obtained from the first and second passages remained healthy, but one of the fowls of the third passage developed slight lesions in the mouth on the tenth day. The fowls inoculated intravenously with material obtained from the fourth and fifth passages developed fairly severe lesions which eventually cleared up, while the fowls of the seventh, eighth, and ninth passages became severely infected, and some died. The in-contact fowls of the sixth to the ninth passages contracted infection.

The lesions produced were similar in appearance to those of fowl pox, but were softer and more greasy to the touch.

The results are shown in Table VII.

TABLE VI.

SHOWING COMPARATIVE RESULTS OBTAINED FROM TESTING VACCINATED AND NON-VACCINATED FOWLS ON THE COMB AND IN THE MOUTH WITH VARYING DILUTIONS OF FOWL POX VIRUS.

Dilution of Fowl Pox Virus	1/5,000				1/10,000				1/15,000				1/20,000			
Day	*7th*	*14th*	*21st*	*28th*	*7th*	*14th*	*21st*	*28th*	*7th*	*14th*	*21st*	*28th*	*7th*	*14th*	*21st*	*28th*
Vaccinated fowls tested on comb	345 —	—	—	—	1791 ++	+	+	—	872 ++	+	—	—	346 +	—	—	—
Control fowls tested on comb	433 ++	+++	+++	+++ g.	413 +	++	+++	+++	439 ++	+++	+++	+++	409 —	++	+++	+++ g.
Vaccinated fowls tested in mouth	353 +	+++	++	—	89 ++	++	+++	+	254 ++	+	—	—	244 +	—	—	—
Control fowls tested in mouth	417 +	+++	+++ g.	+++	443 +	+++	+++ g.	+++	419 +	++	+++	+++	444 +	++ g.	++	+++

TABLE VI.—*Continued*

Dilution of Fowl Pox Virus	1/30,000				1/50,000				1/100,000				1/500,000			
Day	*7th*	*14th*	*21st*	*28th*	*7th*	*14th*	*21st*	*28th*	*7th*	*14th*	*21st*	*28th*	*7th*	*14th*		*28th*
Vaccinated fowls tested on comb	343 +	—	—	—	88 ++	+	—	—	242 ++	+	—	—	1,840 ++	++	+	++ g.
Control fowls tested on comb	445 ++ g.	++	++	+++	407 +	++ g.	++	+++	412 +	++	++ g.	+++	435 +	++	++	+++ g.
Vaccinated fowls tested in mouth	342 ++	+	—	—	240 ++	+	—	—	265 ++	+	—	—	241 +	+	+	—
Control fowls tested in mouth	415 +	++	++ g.	+++	442 +	++ g.	++	++	449 ++	++	++ g.	+++	423 —	—	+	+++

\+ = one small lesion ; ++ = several lesions ; +++ = numerous lesions ; g = generalised infection.

TABLE VII.

PASSAGE OF PIGEON POX VIRUS THROUGH FOWLS.

Pigeon Pox Virus.

Passage	*Result*	*Comb*	*Intravenously*	*Result*
1	++	1395	283	—
	—	Control	290	—
			Control	—
2	+	1502	313	—
	—	Control	322	—
			Control	—
3	++	1569	525	+ m.
	—	Control	450	—
			Control	—
4	+++	1559	561	+ m.f.
	—	Control	554	+ m.
			Control	—
5	+++	1641	365	+ m.
	—	Control	405	++ c.f.
			Control	—
6	+++	1720	357	++ m.c.
	—	Control	361	++ m.
			Control	+ m.
7	+	1823	382	+++ m.c.f.
	—	Control	402	++ c.
			Control	++ m.
8	+++	150	275	+++ c.m. Died
	+ c.m.	Control	273	+++ c.m.
			Control	++ c.m.
9	+++	106	279	+++ c.m.f. Died
	+ c.	Control	284	+++ c.m.
			Control	+++ c.m.

m = mouth ; c = comb ; f = face.

It is evident from these results that the virulence of pigeon pox virus for the fowl can be exalted by passage through fowls. The pigeon pox virus gradually increases in virulence as it becomes adapted to the fowl, and, finally, as shown by intravenous inoculation, it acquires all the characteristics of typical fowl pox virus.

Field Tests.

The vaccine has been subjected to severe laboratory tests, and the results have been so uniformly good that it has been felt there is justification for issuing it for trial under farming conditions.

During the past six months over 50,000 doses have been issued for use in infected flocks, and with one exception the results have been most satisfactory.

As in every instance we were dependent on the owner for diagnosis it is probable that the one unsatisfactory result was due to an error in diagnosis, and not to the vaccine failing to protect against fowl pox.

Conclusions.

1. Pigeon pox vaccine confers a solid immunity on fowls against natural infection with fowl pox.

2. Immunity is fully established about the 14th day after inoculation.

3. The vaccine confers considerable, but not complete, protection against severe artificial infection with fowl pox virus. It prevents generalisation of infection, and the lesions usually clear up quickly ; this is in contrast with the generalisation of infection, accompanied by loss of condition and heavy mortality, which occurs in non-vaccinated fowls.

4. The vaccine does not give rise to any constitutional disturbance, loss of condition, or, as far as has been ascertained, interfere with egg production.

5. Susceptible fowls do not contract infection when kept in contact with fowls exhibiting active pigeon pox lesions on the comb, mouth, or skin.

6. The virulence of pigeon pox virus for fowls can be exalted by serial passage through fowls.

7. In pigeon pox virus we possess a valuable agent for the protection of fowls against natural infection with fowl pox. When employed in conjunction with hygienic measures it should bring about a marked and rapid reduction in the incidence of the disease.

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TYPES OF TUBERCLE BACILLI IN SWINE TUBERCULOSIS.

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Introduction.

This investigation of swine tuberculosis was undertaken to determine the incidence of the different types of tubercle bacilli in a series of pigs found to be tuberculous at the Central Markets, Smithfield, London. The main purpose of the enquiry was to ascertain what proportion of pigs with tuberculous lesions apparently confined to the glands of the head were infected with avian tubercle bacilli. The suggestion of utilising material from the Central Markets was made by Lieut.-Col. T. Dunlop Young, O.B.E., M.R.C.V.S., to whom we desire to express our thanks for his cordial co-operation and for sending us the specimens.

Carcases of pigs are sent to Smithfield from all parts of England and Wales and are there carefully examined for evidence of tuberculous infection. This examination reveals from day to day a varying but always high proportion of carcases affected with tuberculosis, which in most instances appears to be localised in the glands of the head and neck. In the course of the routine inspections Col. Young (1926)* observed wide variation in the macroscopic appearances of the lesions in the glands, and in consequence of this observation he on several occasions made enquiries of the owners of tuberculous pigs about the feeding of the animals. He elicited the information that many of the affected pigs had never been fed on cows' milk or even grazed in contact with cows. Colonel Young thought it probable that in such instances tuberculous fowls which had lived in close association with the pigs were the source of the infection. It is well known that pigs in this country may become infected with avian tubercle bacilli. This was shown conclusively by F. and A. S. Griffith (1911),† and by A. Eastwood and F. Griffith (1914).‡ The latter authors found avian bacilli in no fewer than 27 out of 73 cases in which the tuberculosis was apparently localised in the glands of the alimentary tract.

During the period from January 25th, 1927, to February 18th, 1927, we received submaxillary glands from 103 pigs. The specimens

* Annual Report, Public Health Department, Corporation of London.

† Royal Commission on Tuberculosis, Final Report, Appendix. Vol. III, p. 145.

‡ Reports to Local Government Board on Public Health and Medical Subjects, N.S., No. 91.

were taken on the days of collection in consecutive series from heads sent into the detention room. Although in each case no lesions were found in any other part of the carcase, it cannot be inferred that the disease in every instance was strictly localised, since the viscera were not available for examination.

Method of Investigation.

Owing to a shortage of guinea-pigs we had to rely in the majority of instances upon direct cultivation for obtaining cultures of the tubercle bacilli. The method of procedure was as follows: The surface of the gland was cauterised and a small piece of tuberculous tissue was removed with sterile instruments through the centre of the seared area. This material was ground up in a test tube and made into an emulsion with a few drops of salt solution. A part of the emulsion was treated with potassium hydrate (2·5 per cent. in the mixture) and used to sow egg and glycerine egg tubes, while the remainder in some instances was inoculated subcutaneously into a guinea-pig. A portion of gland from each pig examined was kept in reserve in cold storage for inoculation into a guinea-pig in the event of the direct cultures failing. The cultures obtained were sub-cultured two or three times on egg or serum and then tested on glycerine egg and glycerinated potato. If the cultures exhibited on these media the characters of the bovine type of organism their virulence was not tested on rabbits. If, on the other hand, the cultures differed from bovine bacilli in cultural characters their specific pathogenicity was ascertained.

Results of the Investigation.

Direct cultures were made from the tuberculous submaxillary glands of 103 pigs. In eight cases the cultures remained sterile, and the reserved material which had been kept several weeks in cold storage did not produce tuberculosis in the guinea-pig. Cultures of tubercle bacilli were obtained from 95 cases—in 93 directly from the original material and in 2 instances through the guinea-pig.

Cultural Characters.

From their characters on egg, glycerine egg and glycerinated potato, the cultures could be placed in one or other of three groups. Of the 95 strains 63 were dysgonic and identical in characters with tubercle bacilli of bovine origin; 31 exhibited the characteristic moist, slimy growths of the typical avian tubercle bacillus; the remaining one produced the luxuriant dry coherent growths of the eugonic human type of tubercle bacillus.

Pathogenicity.

(*a*) *Dysgonic Strains.*—Twenty-six of the 63 viruses which yielded dysgonic strains were tested on the guinea-pig by the inoculation in 24 instances of original material and in two instances of cultures; they proved highly virulent for this species.

Four dysgonic strains which in early sub-cultures had produced moist though thin layers on egg (and later proved to be typically bovine) were tested by intramuscular inoculation on birds—three strains on three fowls, and two strains, one of which had also been inoculated into a fowl, on two pigeons. The birds remained well, and when killed four to six months after inoculation showed small necrotic local lesions only. Cultures were sown in some instances, and bovine bacilli were recovered from the local lesions, but not from the liver or spleen.

(*b*) *Strains with Avian Characters.*—The 31 viruses yielding bacilli of avian type were tested either on the fowl, the pigeon or the guinea-pig alone, or on two of these species of animals. The following table gives the number of viruses tested on one and on two species.

Species.	*Number of Viruses Tested.*
Fowl alone	7
Pigeon alone	1
Guinea-pig alone	7
Fowl and pigeon	4
Fowl and guinea-pig	8
Pigeon and guinea-pig	4
	31

Fowls.

Cultures of 19 strains were tested on the fowl, one by intravenous inoculation into two fowls and 18 by intramuscular inoculation each into a single fowl. The two fowls inoculated intravenously, with doses of 0·1 and 0·01 mg., died in 95 and 171 days respectively of general tuberculosis, the livers and spleens being closely beset with caseating tubercles. The duration of life of these fowls was longer than is usual after intravenous inoculation of 0·1 and 0·01 mg. of virulent avian tubercle bacilli. Of the 18 fowls inoculated intramuscularly with doses of 10·0 mg., 14 died in from 23 to 85 days, and one was killed 134 days after inoculation ; all showed general tuberculosis, the livers and spleens containing numerous caseating tubercles, rich in tubercle bacilli. The remaining three fowls tested with different viruses, when examined post-mortem 87, 111, and 161 days respectively after inoculation, showed local necrotic lesions only.

Further experiments were performed with the three latter viruses. Two strains were inoculated intramuscularly, each into a second fowl, and produced general tuberculosis with numerous bacilli in the lesions. We conclude, therefore, that these strains were fully virulent, and that the slight results in the first experiments were due to low vitality of the cultures used.

The third strain was retested by intravenous inoculation of two fowls, with doses of 0·1 and 0·01 mg. These fowls remained well and were killed 167 and 171 days later respectively. No tuberculous lesions were found in the first fowl and only a few scattered caseating nodules in the lungs of the second. A culture of avian type was obtained from a lung nodule, and this produced acute tuberculosis in a fowl inoculated intramuscularly. The original culture was tested again on two fowls intravenously, with doses of 1·0 and 0·1 mg. One fowl was killed 102 days, and the other died 101 days after inoculation. There was no macroscopical evidence of tuberculosis in either fowl, and tubercle bacilli in small number were found microscopically in the bone marrow of one only. Cultures from the spleen of one of the fowls yielded two colonies of tubercle bacilli, while tubes sown with marrow from the other remained sterile. The original culture of this virus did not produce tuberculosis in a guinea-pig.

Pigeons.

Nine avian viruses were tested each on one pigeon by intramuscular inoculation of culture. One of the pigeons died in 92 days, while the rest were killed in from 130 to 177 days. In all the pigeons the lesions visible to the naked eye were confined to the seat of inoculation in the pectoral muscles, which showed extensive caseo-necrosis and yielded luxuriant cultures. Evidence, however, of dissemination and of multiplication of bacilli in the organs was obtained by cultivation experiments ; in all but one instance, *i.e.*, the pigeon that died (cultures from which became contaminated), avian bacilli were obtained in culture from the liver or the spleen or both organs (cf. results with bovine strains).

Four of the above nine strains were tested on the fowl as well as on the pigeon with equivalent doses. The fowls all developed general progressive tuberculosis, while, as already mentioned, in the pigeons only local effects were produced.

These comparative experiments show that the pigeon is less susceptible than the fowl to experimental infection with avian bacilli.

Guinea-pigs.

Nineteen of the viruses which yielded cultures of avian type were tested on the guinea-pig. The tests were made with the original tuberculous material in eleven instances and with cultures in eight instances. Except in two cases the original material and the cultures produced the limited local effects which are characteristic of infections with avian tubercle bacilli.

In the two exceptional cases the original material caused the severe caseo-necrotic general tuberculosis characteristic in the guinea-pig of infection with bovine tubercle bacilli, and from one of the guinea-pigs bovine bacilli were obtained in pure culture. On the other hand, the cultures in these two cases obtained on glycerine egg directly from the original pig glands were pure cultures of avian bacilli, not virulent for the guinea-pig and virulent for the fowl. From these results we

conclude that the submaxillary glands of the two pigs contained bovine as well as avian bacilli.

The results of the experiments on animals with the avian viruses may be summed up as follows :—

Except with three viruses, the results of the experiments with original material or culture were in accordance with the avian cultural characteristics of the bacilli isolated.

Two of the exceptional viruses gave results which showed that bovine as well as avian bacilli were present in the submaxillary glands of the pigs.

The remaining exceptional virus yielded pure cultures of avian bacilli which, however, were less virulent for the fowl than standard avian viruses. The culture apparently acquired full virulence after a single residence in the body of a fowl.

(*c*) *Eugonic Strain.*—The submaxillary gland of one pig yielded a culture which was identical in cultural characters with the eugonic human type of bacillus. The culture was tested subcutaneously on a rabbit in a dose of 20·0 mg. and produced a local abscess only.

The Macroscopic Characters of the Primary Glandular Lesions Caused by the Different Types of Tubercle Bacilli.

The great majority of the submaxillary glands of the pigs which were infected with bovine tubercle bacilli were conspicuously enlarged and composed wholly or in part of firm caseating or caseo-calcareous substance surrounded by fibrous tissue. On the other hand, in some 14 instances of bovine infection the glands were little if at all enlarged and contained discrete caseous gritty tubercles aggregated here and there into small masses ; these foci readily shelled out from thin fibrous capsules.

The glands from which avian tubercle bacilli were obtained in pure culture were, with one exception, not appreciably enlarged, and showed discrete, sometimes aggregated, caseo-calcareous tubercles similar to those occasionally produced by bovine tubercle bacilli. The exceptional gland was definitely enlarged and beset with caseo-calcareous tubercles, ranging in size up to that of a barley grain.

The gland infected with human tubercle bacilli was not enlarged and contained scattered caseo-calcareous tubercles.

This comparison of the tuberculous lesions produced by the different types of tubercle bacilli indicates that when the submaxillary glands of a pig are much enlarged and extensively tuberculous the infection was probably caused by bovine tubercle bacilli.

When, on the other hand, the glands are little if at all enlarged and contain discrete lesions, one cannot decide from macroscopic appearances alone whether the infection was due to bovine, human or avian tubercle bacilli.

Microscopical Examination of Smear Preparations.

Eastwood and F. Griffith (1914) observed that " the number of bacilli found in smear preparations from the affected glands was often an important indication of the nature of the bacillus. In a gland which, macroscopically, suggested avian infection, the presence of numerous bacilli gave support to this suspicion, which was confirmed generally, though not invariably, by subsequent investigation ; but scarcity of bacilli, though usually associated with mammalian cases, was found to be inconclusive, as this condition also occurred in some of the avian infections." We examined smear preparations from all our cases, and our results agree with those of Eastwood and F. Griffith.

TOTAL NUMBER OF CASES OF NATURAL TUBERCULOSIS IN SWINE IN WHICH THE TYPE OF TUBERCLE BACILLUS HAS BEEN DETERMINED.

ENGLISH STATISTICS.

Authors.	*Number of Cases.*	*Types of Tubercle Bacilli Found.*			
		Bovine.	*Human.*	*Avian.*	*Two types.**
A. S and F. Griffith ...	59	50	3	5	1 (bov. and av.)
A. Eastwood and F. Griffith ...	78	49†	1	26†	2 (1 bov. and av.) (1 bov. and hum.)
A. S. Griffith ...	26	16	—	10	—
R. L. Cornell and A. S . Griffith	95	63	1	29	2 (bov. and av.)
	258	178	5	70	5 (4 bov. and av.) (1 bov. and hum.)

* In the two cases in Eastwood and Griffith's series the bovine strains were obtained from submaxillary glands, the avian from mesenteric glands, and the human from liver. In the other three cases bovine and avian bacilli were associated together in the submaxillary glands.

† The virulence of two of the bovine and two of the avian strains was relatively low ; one avian strain was atypical also in cultural characters.

The Frequency of Avian Infections in Swine.

It will be seen from the table that in three series of tuberculous pigs avian tubercle bacilli alone were responsible for the lesions in from 30 to 38 per cent. of the cases. In two of these series the material investigated was, with six exceptions, taken from pigs in which the disease appeared at the slaughter-house to be confined to the lymphatic glands of the alimentary tract. In the third series there was no selection of cases, but the pigs had passed a previous inspection in the country ; it is therefore probable that in the majority of these cases the tuberculosis was limited to the glands of the alimentary tract.

The results show that in approximately one-third of the cases

of apparently localised tuberculosis in swine the disease was caused by avian tubercle bacilli.

The following figures, quoted from Eastwood and F. Griffith's report on localised tuberculosis in swine, give some information as to the frequency of avian tuberculosis in pigs. During the period between January 25th, 1911, and January 16th, 1913, 24,144 pigs were slaughtered at the Brighton Borough Abattoir, and were specially examined by Mr. Cuckney, the Superintendent of the Abattoir. Of this number 209 were found to be affected with generalised tuberculosis, 393 with slightly disseminated tuberculosis, and 59 with localised tuberculosis. The 59 cases of localised tuberculosis and seven of the cases of slight dissemination were investigated bacteriologically. Of the 59 localised cases eight proved not to be tuberculous, seven though anatomically typical of tuberculosis did not yield cultures, 17 yielded avian bacilli, while from the remainder, as well as from the seven cases of slight dissemination, bovine tubercle bacilli were obtained.

Excluding the non-tuberculous cases and assuming that none of the cases of generalised tuberculosis was due to avian bacilli, the proportion of avian infections was 2·6 per cent. of the tuberculous cases (that is, 17 out of 646).

Conclusions.

1. Natural tuberculosis in swine may be the result of infection with bovine, avian or human tubercle bacilli.

2. The chief cause of swine tuberculosis is the bovine tubercle bacillus.

3. The avian tubercle bacillus is responsible for a considerable proportion of the cases in which the tuberculosis is apparently confined to the lymphatic glands of the alimentary tract.

4. These findings corroborate those of other workers in America and on the Continent of Europe, and support their recommendation that pigs and poultry should not be allowed to run together over the same ground.

PRELIMINARY REPORT ON AN OUTBREAK OF FOWL PARALYSIS IN ENGLAND.

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Fowl paralysis has hitherto not been recorded as occurring in the British Isles.

This disease was first described by Marek (1907), who observed it in Austria. Its occurrence in the United States of America was recorded by Kaupp (1921), who had first seen it in 1914. Van der Walle and Winkler-Junius (1924) described an epizootic in the Netherlands which they had observed three years earlier. Thomas (1928) recorded its appearance in South Africa. Pappenheimer, Dunn and Cone (1929), in their recent extensive communication, state that it is widespread in the United States of America, and probably exists also in South America.

The following brief clinical and pathological description is based on the work of Doyle (1926, 1928), Dobberstein and Haupt (1927), Thomas (1928), and Pappenheimer, Dunn and Cone (1929), who have made detailed investigations.

Clinical Description.

All breeds of fowls and both sexes appear to be equally susceptible. The disease often appears suddenly, and is especially prevalent in the autumn. It persists and becomes endemic in certain areas, cases occurring year after year in spite of all efforts to eradicate it. It is most prevalent and severe in birds from three to eight months of age, but is seen occasionally in younger birds. Doyle (1928) mentions that a bird 47 days old and several less than two months old were affected. Cases have been recorded in birds over one year old.

The development of clinical signs is usually slow and the course prolonged, although acute cases with death in a few days have been recorded by Thomas (1928). Affected birds may live over three months if carefully attended, but seldom recover. Except in the later stages appetite is maintained, although there is often emaciation. The severity and distribution of paralysis varies in different birds in the same outbreak. In mild cases or early stages there may be only drooping of one wing. In more advanced cases there may be lack of co-ordination in walking, causing a stilted or jerky gait. Paralysis is generally spastic, and is sometimes accompanied by increased reflexes and clonic spasms. The most extreme stage is one of complete prostration. The birds lie helpless and immobile, but if handled may struggle vigorously ; all desire for food and water is lost, and the bird

takes no interest in its surroundings. At this time diarrhœa or incontinence is often seen. Doyle (1928) states that dyspnœa and wry-neck are very common signs in all stages, as is also partial or total blindness due to iritis, which has been said (Doyle, 1926) to have a flock association. It is doubtful if it is an essential part of the disease. One or both eyes may have a dull glazed appearance, the iris is fixed and unresponsive, and has changed in colour to slate-grey. Pappenheimer, Dunn and Cone (1929) mention two experimental cases in young cockerels where spermatogenesis was delayed. The possibility of the existence of infection without clinical signs has been indicated by these authors, who found microscopic evidence of the disease in birds which they examined as controls.

General Pathology.

The commonest lesion is enlargement of the posterior roots of the spinal nerves and their ganglia. They are abnormally translucent, and have a slight yellowish or brownish tinge. Sometimes extension into the cord of the translucent or yellowish tissue may be seen. The enlargement of the ganglia is in most cases unilateral. Pappenheimer, Dunn and Cone (1929) record one case in which there was enlargement of the acoustic nerve, and another in which the trigeminal nerve was thickened. As a rule no gross lesions are seen in the brain, cord, or membranes. The peripheral nerves, particularly those of the brachial plexus and the sciatic nerves, are frequently thickened, showing the same translucency and yellowish discolouration as the posterior root ganglia. The authors referred to also found lymphomatous tumour-like masses in 10 per cent. of the birds which they examined. They state that these lymphomata appear to originate in the ovary, none having been found in males, but there were only four males among the sixty cases examined. Nodules were seen in the liver, kidneys, lungs, adrenals, and muscles in some cases, and in one instance the spinal cord had been invaded, but there was no certainty that these were true metastases. Nor is there certainty that this type of lesion is due to the same cause as the lesions of the nervous system.

The microscopic appearances in diseased birds have been studied by Marek (1907), Doyle (1926, 1928), Dobberstein and Haupt (1927), Thomas (1928), and by Pappenheimer, Dunn and Cone (1929). In the brain, mononuclear cells are commonly found in small isolated nodules and sometimes in accumulations round the small blood-vessels. Many of these cells are of the small lymphocytic type, while others are larger, with more vesicular nucleus, and in these mitoses are frequently found. Cells similar to mammalian plasma cells are also numerous. In the cord similar perivascular infiltration with mononuclear cells is seen, but the most notable changes occur around the entrance of the affected nerve roots. The nerve roots themselves show dense infiltration with the types of cells referred to, so that the nerve tissue is apparently obliterated. This infiltration extends into the contiguous parts of the cord and occasionally the grey matter is involved. In the posterior root ganglia the nerve cells sometimes

show degenerative changes, apparently secondary to the infiltrative process. The nerve trunks in the brachial plexus and sciatic nerve are sometimes affected, and show typically massive cellular infiltration with, in some cases, degeneration of the nerve fibres. The cranial nerves and splanchnic nerves and ganglia were occasionally affected in the birds studied by Pappenheimer and his colleagues. The iritis which is sometimes present is possibly not essentially the same as the other lesions ; the cells are more often of the polymorphonuclear and eosinophile leucocyte types. Nodules of mononuclear cells have also been observed in the liver, kidney, and adrenals. In the lymphomatous nodules in the ovaries of six birds examined by Pappenheimer, Dunn and Cone, the mononuclear cells showed a marked tendency to invade the ovarian tissue and were of the larger type, and mitotic figures were numerous.

In the studies of Thomas (1928) and Pappenheimer, Dunn and Cone (1929) the cellular accumulations found in the nervous tissue and occasionally other organs varied from small foci of lymphoid cells to large sarcoma-like new growths.

Aetiology.

The aetiology of this disease is still obscure. Repeated attempts by several investigators to isolate a causative organism have been unsuccessful. Kaupp (1921) inoculated heart blood, liver, kidney, spleen, various parts of the brain and cord, especially of the affected segments, into rabbits, guinea-pigs, and young and year-old fowls without success. May, Tittsler and Goodner (1924), Doyle (1926) and Thomas (1928) carried out similar experiments without any definite results. Pappenheimer, Dunn and Cone (1929) were unable to discover any infecting organism in smears and sections of brain, spinal cord, peripheral nerves, and heart blood. Sections were stained by the Gram-Weigert method, toluidine blue, Giemsa's method, Wright's stain, Levaditi's method, and Jahnel's modification of Levaditi's method for spirochætes. No intranuclear or cytoplasmic inclusion bodies were seen. The last authors also made numerous attempts to obtain cultures of organisms, using various media and methods, but without success. They also came to the conclusion that coccidia were not the cause of the disease.

Avitaminosis has been excluded as the possible cause because numerous outbreaks have occurred in flocks where the diet was all that could be desired.

Examination of blood smears and the absence of the characteristic enlargement of the spleen and liver distinguishes the disease from leukæmic conditions. The evidence brought forward so far in support of the theory that the disease is caused by a virus is not very conclusive. Van der Walle and Winkler-Junius (1924) fed a hen with muscle from an affected hen. The bird became atactic after five months. The blood of this hen kept anœrobically for 18 days, at 37° C., was injected into a healthy bird. A bouillon culture was made from the blood,

filtered through a Chamberland filter, and injected intravenously into another hen. This bird showed symptoms of paralysis after two months, but recovered in two weeks. Another hen, injected intraperitoneally with blood of a diseased bird, developed paralysis after 46 days. Five birds kept in the same pen with affected birds failed to develop the disease. These authors concluded that the disease was an infection due to a filtrable virus.

Pappenheimer, Dunn and Seidlin (1929) attempted to transmit the disease by the inoculation of young chicks with unfiltered affected tissue. Twenty-six per cent. of the inoculated birds developed characteristic symptoms and lesions, but 7 per cent. of uninoculated control birds also developed the disease, even when they were kept isolated from the inoculated birds. Only one filtration experiment was made, and this was negative. In another experiment ten birds were inoculated with material which had been mixed with 50 per cent. glycerol and kept in the ice-box for nine days. One developed paralysis after 106 days, and on post-mortem examination extreme gross and microscopic lesions of the disease were found. In two others there were no clinical signs or gross lesions, but microscopic lesions were found in the peripheral nerves. In the discussion of their result, they point out the difficulties encountered in experiments on this disease, viz., the insidious development, the long latent period between inoculation and development of symptoms or frank lesions, the apparent resistance of many individual birds, the occurrence of lesions of every grade of intensity ranging from insignificant histological infiltration to massive tumour-like enlargements, the difficulty of obtaining control material free from lesions, all rendering the experimental study of the disease laborious and conclusions hazardous.

History of the Present Outbreak.

Our attention was drawn to the occurrence of this outbreak by Mr. G. C. Marginson, M.R.C.V.S., who consulted us with reference to the diagnosis of a disease of an unusual character in a flock of poultry.

Fowl paralysis was suspected as soon as the first three pullets were received, and diagnosis was confirmed when the striking gross and microscopic lesions were found on post-mortem examination.

We are indebted to Mr. Marginson for the following information. In the autumn of 1927 the owner of the affected flock bought 60 cockerels out of a lot of 800 sold at a dispersion sale of a poultry-breeder's stock. Of these 60 cockerels 20 died after showing signs of what was apparently fowl paralysis ; the remaining 40 were mated in January, 1928, with healthy pullets. A few of their progeny showed similar signs, and the surviving pullets were mated with fresh healthy cockerels in January, 1929. The disease first assumed alarming proportions in August, 1929. In the period from August to December, 1929, 700 to 800 birds died after showing signs of fowl paralysis. The majority of these birds were young pullets, grand-daughters

of the cockerels bought in 1927, but some were young pullets hatched from eggs bought from other breeders. A few cases occurred among the older pullets. The signs shown in this outbreak correspond closely with those described by the authors previously mentioned. Iritis was very common and sometimes the only evidence that a bird was affected. " Wry-neck," hanging of the head to one side, and walking backwards were also prominent features. Emaciation was the rule with advanced cases, the average weight of the pullets examined being about 2 lbs. 6 ozs.

Interference with egg-production has been a striking feature. Laying pullets stopped laying, and young pullets did not commence to lay. It was noticed that affected pullets had an immature appearance, the comb not being fully developed even at eight or nine months of age.

Ten pullets were examined at the laboratory. Aerobic and anœrobic blood cultures were made from two birds, but no organisms were isolated. Three of the pullets died, the others were killed by bleeding from the jugular vein. A careful post-mortem examination was made in every case, and from the majority pieces of organs were kept for histological study. The brain and spinal cord with attached roots were then carefully dissected out and examined. Pieces of the brain, the spinal cord in the upper thoracic and lumbar regions, and in some cases the peripheral nerves, were fixed in 10 per cent. formol saline for histological examination. Sections were stained by hæmalum and eosin, Mann's, Giemsa's, Gram-Weigert's, and Ziehl-Neelsen's methods. A note of the histological findings in the nervous system is included in the following notes on each of the ten birds.

Pullet 1.—*White Wyandotte-Leghorn crossbred,* 8 *months old. Received on November* 30*th,* 1929.

Emaciated; weight 2 lbs. 4 ozs. Unable to stand, lethargic, but could be roused by a loud noise. No desire for food and water. Struggled if handled. Found dead on December 3rd, 1929.

Post-mortem examination.—No lesions were noticed in the brain and spinal cord, and the internal organs seemed to be normal. The ovary had not functioned.

Microscopic examination.—Slight perivascular infiltration of small mononuclear cells in the pons and a similar appearance in the white matter of the cord in thoracic region were noted. Nothing abnormal was seen in sections of the cerebrum, cerebellum, lumbar region of cord, or in the liver, kidney, or spleen.

Pullet 2.—*White Wyandotte-Leghorn crossbred,* 8 *months old. Received on* November 30th, 1929.

Emaciated ; weighed 2 lbs. 2 ozs. Able to walk, but in a jerky, stilted fashion. Appetite good. Found dead on December 17th, 1929.

Post-mortem examination.—No obvious lesions were seen in the brain, cord, roots and nerves, or internal organs. Histological examination was not carried out.

Pullet 3.—*White Wyandotte-Leghorn crossbred. Received on December 6th,* 1929.

Emaciated ; was unable to walk, always lay on its left side. There was almost continuous jerking movements of the legs. On examination of the blood the cell counts and hæmoglobin content were found to be normal. Killed on December 23rd, 1929.

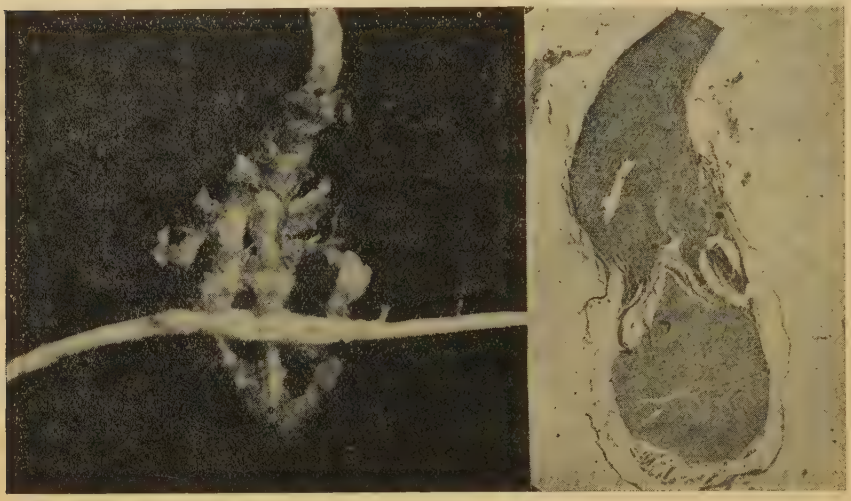

FIG. 1. FIG. 2.

FIG. 1.
Pullet 4. Cord and bracial plexus. Natural size.
FIG. 2.
Pullet 4. Cord and second thoracic root on left side. X 10.

Post-mortem examination.—No gross lesions were seen in the viscera. The posterior roots of the spinal cord in the upper thoracic region on the left side were greatly thickened, and yellowish-brown in colour. The nerves of the left brachial plexus were also thickened, and yellow in colour. No gross lesions were found elsewhere in the nervous system.

Microscopic examination.—In the cerebrum there was slight perivascular infiltration with small round cells ; the pons and cerebellum showed nothing abnormal. The posterior roots on the left side of the cord showed dense infiltration with mononuclear cells of the large type. Many mitotic figures were seen. The ganglion cells were few in number and degenerated. The nerve trunks of the left brachial plexes showed the same appearance as the roots, but the infiltrating cells were smaller in type.

Pullet 4.—*Rhode Island Red. Received on December* 24*th*, 1929.

This bird was mature, and had laid eggs before it became affected. Prostrated, emaciated, and lethargic. No desire for food and water. Lay on its left side ; the left leg and left side of the body were denuded of feathers, and the skin was thickened and excoriated. The left wing drooped and was not freely movable. The cornea of the right eye was opaque. Killed some hours after it was received in the laboratory.

Post-mortem examination.—In the skin covering the left pectoral muscles there was a round tumour, rather flattened, about the size of a penny, with

a well-defined edge, and not attached to subcutaneous tissue. When cut into it was firm and yellow. The muscles of the left wing were atrophied, soft, and œdematous. The posterior roots on the left side in the thoracic region of the cord and the nerves of the left brachial plexus were greatly thickened, firm, and brown (Fig. 1). The viscera appeared normal, and the ovary had functioned.

Microscopic examination. No changes were noted in the cerebrum, cerebellum, or pons. In the upper thoracic region the left posterior roots showed massive infiltration with large round cells (Fig. 2). Many mitotic figures were seen (Fig. 3), and the infiltration extended into the white and grey matter of the cord on the same side. In the cord itself well-marked " cuffing " of the vessels was noted. The thickened trunks of the brachial plexus on this side showed the same type of cellular infiltration as in the root (Fig. 4). The iris of the right eye showed infiltration with eosinophile and mononuclear cells. In the anterior chamber there was an exudate composed mostly of eosinophile polymorphonuclear leucocytes, and the cornea showed slight infiltration with the same type of cell. Histological examination of the tumour of the skin showed appearances identical with those in the thickened nerve root (Fig. 5). The muscles of the skin were invaded by the cells of the tumour.

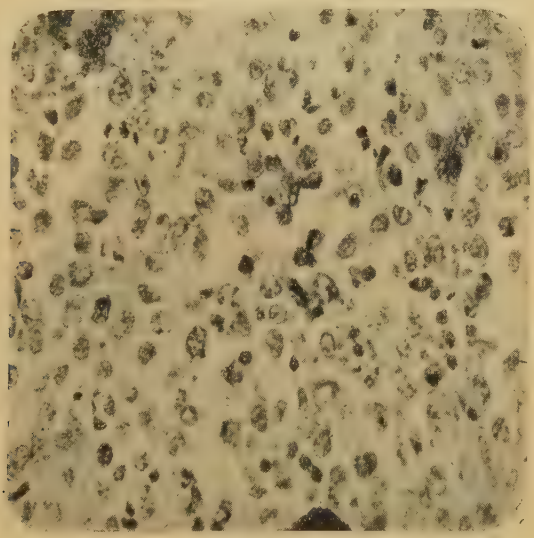

FIG. 3.

Pullet 4. High power of enlarged root shown in Fig. 2. Mitotic figures. X 500.

Pullet 5.—Rhode Island Red. Received on December 24th, 1929.

Very emaciated, and had no desire for food and water. The bird lay helpless, and was lethargic, but would struggle violently if handled. The right side of the body and the right leg were denuded of feathers, the skin thickened and encrusted. The right wing drooped. This bird was found dead on December 27th, 1929.

Post-mortem examination disclosed no gross lesions in the internal organs or nervous system.

Microscopic examination.—The brain showed no microscopical lesions. In the anterior root in the upper thoracic region there was slight infiltration with small round cells. The posterior roots showed similar changes and, in addition, " cuffing " of the vessels.

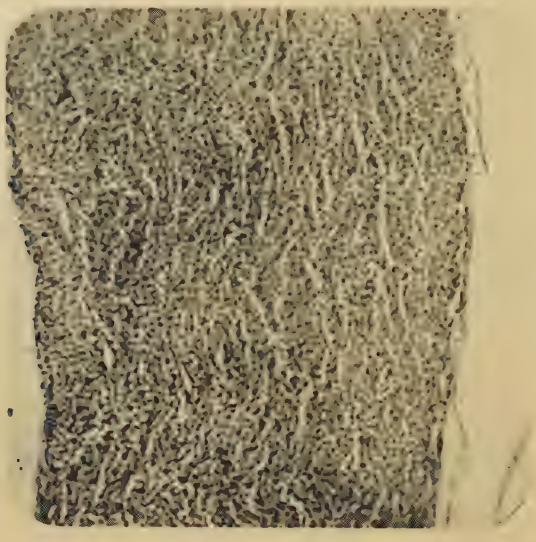

FIG. 4.
Pullet 4. Nerve trunk brachial plexus, left side. X 100.

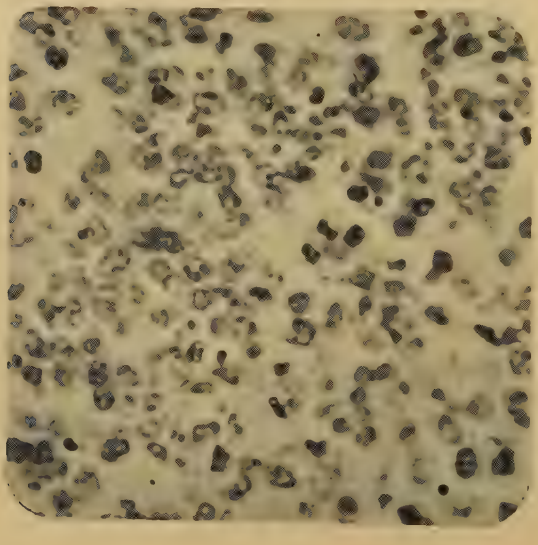

FIG. 5.
Pullet 4. Skin tumour showing mitotic figures. X 500.

Pullet 6.—*White Wyandotte crossbred, about* 8 *months old. Received on November* 30*th*, 1929.

Was emaciated, weight being 2 lbs. 10 ozs. The bird was able to walk quite well, but was completely blind ; the iris and anterior chamber of both eyes were clouded. The head and neck were continually twisted to the left side. The bird would eat and drink if food and water were placed near it. It was kept alive for two months, during which time its condition remained unchanged. Killed on January 22nd, 1930.

Post-mortem examination.—The brain and internal organs appeared to be normal. There was a very slight thickening of the posterior roots of the spinal cord in the cervical and thoracic regions.

Microscopic examination.—There was well-marked perivascular infiltration in the mid-brain. The posterior root ganglia in the thoracic region showed slight infiltration with small round cells. A similar change was noted in a sympathetic ganglion near the left adrenal gland. One iris on examination showed infiltration with cells resembling mammalian plasma cells. No changes were found in the optic nerve or retina.

Pullet 7.—*White Wyandotte crossbred, about* 8 *months old. Received on December* 6*th*, 1929.

The bird was in poor condition, but not markedly emaciated. It was able to walk quite well, but the left leg was raised high and brought forward with a jerky movement, brushing the feathers of the left wing, which drooped slightly. The feathers of the left wing were worn away by the friction. The appetite was quite good, and the bird was kept alive for two months, during which time the disease did not seem to progress. Killed on January 27th, 1930.

Post-mortem examination.—The internal organs and the brain appeared normal. The posterior roots of the spinal nerves on both sides of the cord

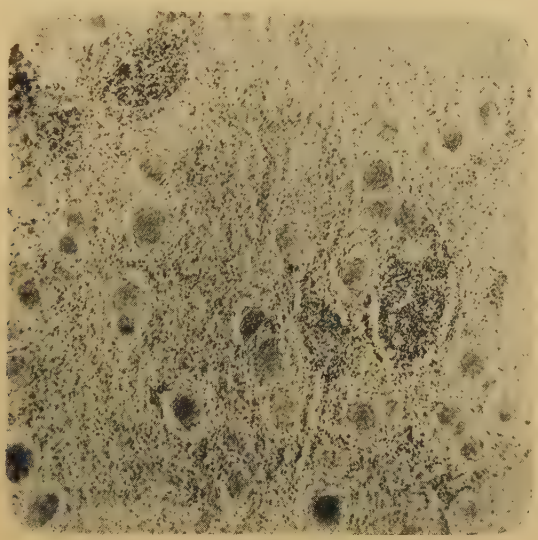

FIG. 6.
Pullet 7. Posterior root ganglion, thoracic region, showing small round cell infiltration. X 50.

in the dorsal and lumbar regions were slightly thickened. The ganglia of the acoustic and facial nerves seemed to be slightly swollen.

Microscopical examination.—The cerebrum showed very slight round-cell perivascular infiltration ; this was more marked in the vessels of the meninges. In the thoracic region of the cord there was round-cell infiltration of the vessels at the junction of the roots and cord on both sides. At this level the posterior root ganglia showed collections of small round cells (Fig. 6). No infiltration was observed in the sciatic nerve from the left leg.

Pullet 8.—*White Wyandotte crossbred*, 8 *months old. Received on December* 6*th*, 1929.

Paralysis was well advanced in this case. The bird walked with difficulty, showing marked inco-ordination of movements. Both wings drooped, and the neck was twisted to one side. Appetite was good, but the bird was emaciated. Killed on January 8th, 1930.

Post-mortem examination.—Nothing abnormal was noted in the internal organs. The ovary had not functioned. A small nodule about the size of a millet seed was present on the sympathetic nerve near the gall-bladder. The brain, cord, and eyes, appeared normal. The posterior roots of the spinal nerves in the thoracic region and the brachial plexus on both sides were slightly thicker and greyer than normal, particularly on the right side. The lumbar roots were also slightly affected, especially on the left side.

Microscopic examination.—No changes were noted in the brain. In the thoracic region the posterior roots on both sides showed moderate infiltration with small round cells, and " cuffing " of the vessels was well marked in the white matter of the cord near the junction with the roots (Fig. 7). The trunks of the lumbar plexus on the right side showed changes similar to those in the posterior roots of the thoracic region. Similar infiltration was observed in the enlarged sympathetic ganglion from the region of gall-bladder.

Pullet 9.—*Crossbred Wyandotte*, *about* 8 *months old. Received on January* 3*rd*, 1930.

The appetite was good, but the bird was in poor condition. It walked with difficulty, the wings drooped, and the feathers were worn away on the right side of the body. Killed January 9th, 1930.

Post-mortem examination.—A tumour about the size of a penny and $\frac{1}{4}$-inch thick was found in the skin of the neck on the left side. The tumour was firm, had a well-defined edge, and was not adherent to the underlying muscle. The cut surface was yellow. Nothing abnormal was noted in the brain or internal organs. The posterior roots on the left side of the cord in the upper thoracic region were slightly thickened. Similar appearances were seen in the roots on both sides in the lumbar region. The ganglion in the left acoustic nerve was thickened and brownish.

Microscopic examination.—The tumour in the skin showed the same histological appearances as that found in Pullet 4, but few mitotic figures were seen. There was very slight perivascular infiltration in the cerebrum, and in the thickened acoustic nerve ganglion there was moderate infiltration with large round cells. The sections of the thoracic nerve roots showed slight infiltration with cells of small type.

Pullet 10.—*Black Pullet, about* 1 *year old. Received on February 4th*, 1930.

This bird first showed signs of paralysis a few days before it was received. On arrival at the laboratory it was lethargic and unable to stand. Appetite was good, and the bird was not much emaciated. Died on February 14th.

Post-mortem examination.—The internal organs appeared to be normal The cerebrum, cerebellum, and meninges were markedly congested. The acoustic ganglion on the left side was enlarged, and the fifth cranial nerve on the right side was thickened and greyish in colour. The ganglia in the nerve roots in the cervical region of the cord were slightly thickened, and the sympathetic trunk on the left side in the lower cervical region was markedly thickened. In the upper thoracic region the roots were markedly enlarged, especially on the left side, and the thickening extended into the cords of the brachial plexus. The roots and trunks of the lumbar and lumbosacral plexuses on both sides were thickened, more particularly on the left. On this side a soft greyish-pink non-capsulated tumour mass, 1·2 by 1·5 cm. in extent, was found around the roots of the lumbosacral plexus (Fig. 8). This mass had extended on to the anterior surface of the vertebral column, and was in close contact with the kidney on the side. The sciatic nerve was enlarged, greyish, and congested, and the thickening extended 6 cm. along the nerve from its origin.

Microscopic examination.—In the cerebrum and midbrain perivascular infiltration was present around a few vessels. Examination of the cord and roots at various levels showed cellular infiltration which corresponded to the distribution of the lesions noted above. The infiltration in the fifth nerve was of the small-cell type. The loose tissue between the affected sympathetic trunk in the lower part of the cervical region and the thickened roots at this level showed invasion by large mononuclear cells. The tumour in the lumbosacral region was very similar in appearance to those noted in the skin of pullets 4 and 9. The cells of the tumour were of the same type as those within the nerve roots which could easily be recognised within the

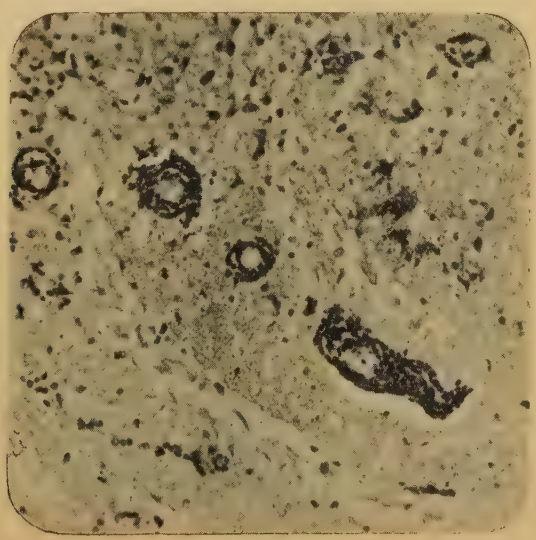

FIG. 7.

Pullet. 8. Spinal cord, thoracic region, showing perivascular infiltration. X 100.

tumour mass (Fig. 8). Numerous thin-walled vessels were present throughout the tumour, and the cord on the same side showed well-marked perivascular infiltration extending into the grey matter (Fig. 9). The cells in the more distal part of the sciatic nerve were rather smaller in type than those present near the origin of the nerve.

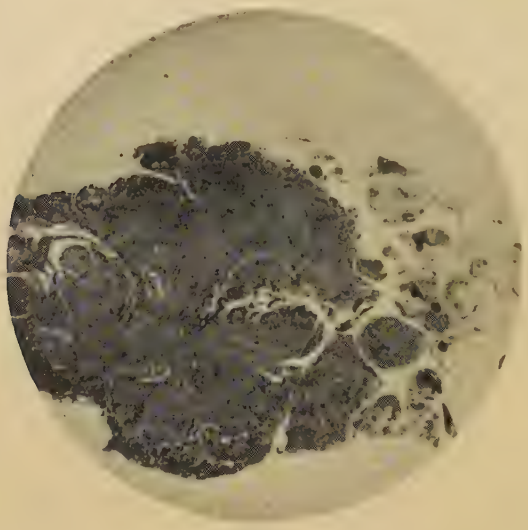

FIG. 8.
Pullet 10. Tumour and nerve trunks, left lumbosacral plexus. X 10.

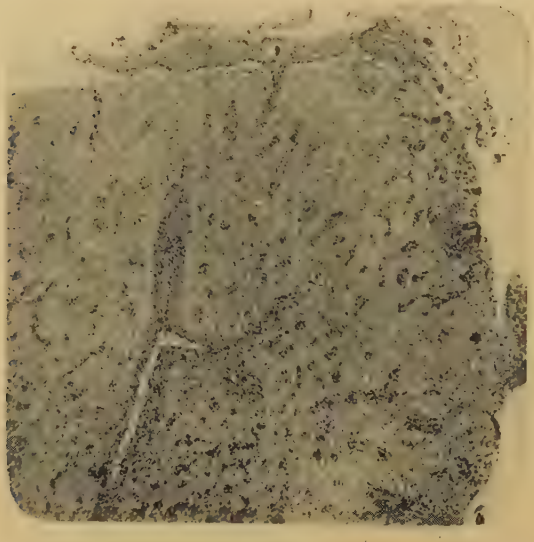

FIG. 9.
Pullet 10. Lumbosacral region of cord, left side, showing extension of cellular infiltration along the vessels. X 35.

It will be seen from these notes that gross lesions in the brain were not found in any of the ten fowls, and only in a few was slight perivascular infiltration with small round cells seen. The lesions in the nerve trunks and nerve roots were not symmetrical. The microscopical appearance varied in different birds, and in different lesions in the same bird. In the very extensive thickening of the nerve roots noted in pullets 3 and 4 the cells were large and numerous mitotic figures were present ; the microscopic appearances were such as to suggest sarcomatous change. This proliferation of round cells of the large type was found extending into the white matter on the same side of the cord, and was present in the nerve trunks of the brachial plexus of Pullet 4, and also in the acoustic nerve ganglion of Pullet 9, and in the nerve roots and tumour in Pullet 10. Similar collections of cells, some of which showed mitosis, were observed near blood vessels in the loose areolar tissues removed with the nerve trunks from the brachial plexus of Pullet 4, and in a similar situation in Pullet 10. The histological appearance of the tumour present around the nerve trunks in the lumbosacral region of the latter bird suggests that it arose by extension of the proliferative process within the nerves. In other birds the cellular infiltration found in the roots and ganglia was composed mostly of cells of the small lymphocyte type, and occasionally, as in Pullet 3, the infiltration of the large-cell type found in the thickened root gradually gave place to infiltration of the smaller cell type in the nerves of the brachial plexus.

The absence of total paralysis in the wings and legs of birds showing microscopical changes in the nerve roots of the corresponding regions of the cord suggests that the nerve fibres were not destroyed, but merely affected by the pressure of the infiltrating cells. The deposits of mononuclear cells, mostly of the small type, found in the sympathetic ganglia near the adrenal in Pullet 6 and in the ganglion near the gall-bladder in Pullet 8 indicate the widespread distribution of the lesions in the peripheral nervous system.

Histologically, the tumours found in the skin of Pullets 4 and 9 were composed of large round cells similar to those found in the more severe lesions of the nerve roots, and the appearances certainly suggest that these were manifestations of the same disease process. The cellular infiltration noted in the eyes of Pullets 4 and 6 was mostly of polymorphonuclear and eosinophile type, which rather indicates, as Doyle (1926) believed, that although the presence of iritis is not uncommon in this disease it is probably not primarily due to the same etiological factor.

A characteristic feature in the histology of the lesions noted is the combination of cellular proliferation and tumour formation apparently of sarcomatous nature as found in the nerve roots with, in other parts, collections of cells of a small lymphocytic type suggesting granuloma.

Discussion.

The great variety in clinical appearances noted by other authors has also been seen in this epidemic. Iritis seems to have been more

common than is usual, and the arrest of maturity in young pullets and interference with egg production in older pullets have been striking and serious features. The lesions described correspond in their distribution and in the macroscopic and microscopic appearances with those recorded by the authors previously mentioned. The visceral lymphomata noted by Pappenheimer, Dunn and Cone (1929) were not encountered in the birds examined by us. On the other hand, the microscopic appearances of the tumours found in Pullets 4 and 9 were identical with the lesions found in the nerve roots in the badly affected birds. This indicates that the disease process is not confined to the central nervous system and large nerve trunks. It may be noted that, although Pullets 1 and 5 showed advanced clinical signs of the disease, no marked gross or microscopical changes were found in the nervous system—a fact which suggests that the clinical condition could not always be explained by the pathological findings, and might be partly the result of some toxic action.

It has been observed by Doyle (1928) that birds hatched from eggs obtained from an affected flock usually develop the disease. In this connection it is interesting to note that in the present outbreak the disease appeared in a neighbouring farm in birds hatched from eggs obtained from the affected flock. The disease seems also to have become introduced into a flock 15 miles away in the same manner.

Because of the high mortality rate, the loss in egg production, and the tendency to become endemic, this disease is of the utmost importance to poultry breeders.

Although in the case of the present outbreak the disease may have been introduced from abroad, it was first noticed in birds obtained from a breeder in this country, and it is possible that fowl paralysis exists in other parts of England.

We are indebted to Professor Maitland for his helpful advice and criticism during the preparation of this paper.

The photographs are by Mr. H. C. Taylor, Manchester University.

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SELECTIVE MEDIA FOR THE CULTIVATION OF *Bacillus Pullorum* AND *Bacillus Sanguinarium.*

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(Received for publication, December 18th, 1929.)

During the course of post-mortem work, many carcases of chicks are received which, owing to putrefactive changes, are completely unsuitable for the diagnosis of bacillary white diarrhœa, the contaminating organisms which have gained access to the body cavity crowding out the more delicately-growing *Bacillus pullorum.* To overcome this difficulty, certain inhibitive media have been investigated with a view to selecting one which would permit the growth of *Bacillus pullorum* and other members of the typhoid group, and at the same time inhibit the commoner putrefactive organisms. The advantages of such a medium are obvious, as the delay which arises in forwarding chicks from isolated areas during hot summer weather, permitting putrefactive changes to take place, would then cease to be a source of worry. The possible use of such a medium in fæcal examination for *Bacillus pullorum* occurred to the writer, following the work of Stafseth and Mallman,[4] who claimed that a selective and inhibitive medium utilising brilliant green may be used successfully in fæcal examination. With this in view, the following experiments were carried out :—

Routine Methods for the Isolation and Identification of B. pullorum *and* B. sanguinarium.—The existing routine laboratory examination for the diagnosis of bacillary white diarrhœa in chicks consists in the inoculation of agar slopes from the liver, and also from the yolk sac if this is still unabsorbed. In the diagnosis the presence of microscopic lesions are also taken into consideration.

The medium adopted is a standard meat extract broth plus 3 per cent. agar, adjusted to *p*H 7·4.

After 24 hours' incubation at 37° C. the cultures are examined, the character of the growth and whether the culture consists solely of Gram-negative organisms being noted before further identification is proceeded with.

The organism is identified by :—

(1) Fermentation Tests : Litmus milk and 1 per cent. solution in peptone water of lactose, glucose, maltose, and mannite, to which 1 per cent. Andrade's indicator has been added, are inoculated from the culture. The differential effects produced on these media are used to identify organisms of this group.

(2) Agglutination Test : An emulsion of the suspected organism is agglutinated with a known serum prepared from a rabbit, the dilution used being 1-100.

General Experimental Methods Adopted in the Use of Inhibitory Media.

The inhibitory agents are prepared in stock solutions of 1-1,000, a definite quantity being added to fluid agar at a temperature of 60° C. to obtain the necessary dilutions required for the purpose of the experiment on hand. Twenty c.c. of the fluid agar so treated is poured into Petri dishes (diameter 3 ins. to 4 ins.) and allowed to set. Ordinary agar plates are poured and used as controls in each instance.

Fæcal emulsion is prepared by emptying the contents of the posterior part of the bowel into a test tube and adding sufficient sterile normal saline to make a dilution of 1-4 or 1-16, according to the experiment. A little glycerine is added to prevent over evaporation if the fæcal emulsion is retained for any length of time.

A two-millimetre loopful of the fæcal emulsion is placed on the plate and is spread all over with a piece of glass rod bent at right angles ; this may then be used for inoculating a second plate and possibly others without recharging.

The usual period of incubation is 48 hours at a temperature of 37° C., unless where otherwise stated.

Inhibitory Agents.

Amongst the antiseptic substances tried as inhibitory agents were telluric acid, picric acid, thymol, and copper sulphate, but the agents which apparently give the most satisfactory results are members of the aniline group of dyes. More or less detailed observations on the agents tested are appended.

Thymol.

Added to culture media in all dilutions up to and including 1-60,000 inhibited Gram-positive organisms, but permitted many Gram-negative organisms of the coliform group to flourish. *B. pullorum* grew quite well on media so treated, but was crowded out by more vigorously-growing organisms.

Copper Sulphate.

Had an action similar to thymol when added to nutrient media in a dilution of 1-30,000.

Telluric Acid.

A white crystalline substance which possesses a more definite inhibitory action than thymol and copper sulphate and has the characteristic effect of colouring the coliform contaminators a brownish-black colour, which becomes more pronounced with incubation exceeding 48 hours. The optimum dilution of this drug is 1-25,000 ; any stronger dilution has a definitely inhibitive effect on *B. pullorum.*

Picric Acid.

Had an action similar to thymol and copper sulphate when used in a dilution of 1-10,000.

Aniline Group of Dyes.

The antiseptic properties of the members of the aniline group of dyes have been recognised for many years. A special interest is attached to the fact that their toxic action seems to be directed chiefly against Gram-positive bacteria. They have a very effective antiseptic action on wounds, and are sometimes used for the purpose of sterilising the skin prior to an operation. Used in nutritive media, they have a definite inhibitive action, the degree of which depends upon the dye used.

Many workers have recommended the use of crystal violet, basic fuchsin, methylene blue, and neutral red in the preparation of the differential media for the isolation of coliform and typhoid colonies, but it was found that these dyes did not possess selective and inhibitive properties sufficient for the requirements of the investigation.

Conradi and Drigalski have pointed out that certain dyes of the diamino-triphenylmethane group (malachite and brilliant green), when present in suitable concentrations, inhibit most types of coliform bacilli and permit the typhoid group to grow freely.

In this investigation these substances were found to possess a differential action on colonies of *B. pullorum* and *B. sanguinarium*. Their most characteristic property is, however, the total inhibition of Gram-positive organisms.

In a series of experiments in which various dilutions of the different dyes were used brilliant green was found to be superior to the other dyes investigated.

Experiment 1.—To compare the selective properties of various dyes of the aniline group for *B. pullorum* and their inhibitive effect on other organisms.

Fæces from week-old chicks were collected and emulsified 1-4 by the usual method, ·02 c.c. being used for the inoculation.

Acriflavin, safranin, malachite green, methyl green, and brilliant green were the dyes selected for the experiment.

Dilutions of each dye in agar plates were respectively, 1-100,000, 1-200,000, 1-400,000, 1-800,000 and 1-1,600,000. The plates were examined for *B. pullorum* and contaminators after 48 hours' incubation.

The dyes acriflavin, safranin, and malachite green in media showed in all dilutions a heavy contamination of Gram-negative organisms, mostly of the coliform group, *B. pullorum* being crowded out on all the plates. Methyl and brilliant green alone showed effective inhibition.

Methyl Green.—Dilutions of 1-100,000 and 1-200,000 showed a good growth of *B. pullorum* free from contaminating colonies.

With 1-400,000 one cluster of coliform colonies was observed on one plate.

With 1-800,000 a greater number of contaminating colonies was present on both plates, making it difficult to differentiate *B. pullorum*.

With 1-1,600,000 *B. pullorum* was overgrown with contaminators.

Brilliant Green.—Dilutions of 1-100,000 to 400,000 were free from contaminators.

1-800,000 showed three single contaminating colonies.

1-1,600,000 showed on the two plates four and twelve contaminating colonies respectively.

In the dilution of 1-1,600,000 brilliant green plates the contaminating colonies were picked off and stained by Gram's method, but this failed to show colonies of organisms possessing Gram-positive characters.

The duplicate series of plates corresponded in the results very closely to the plates of the experiment in all the dilutions.

Brilliant green when added to a nutrient medium in a dilution of 1-5,000,000 was found to inhibit streptococci and staphylococci for a period of 24 hours, whereas a dilution of 1-1,000 permitted the growth of *B. pullorum*. This organism also grew in a dilution of 1-500, but the inhibitory effect was shown by the rather scanty growth and the pin-point size of the colonies.

Brilliant green thus appeared to be the most promising inhibitory agent as yet examined, and certainly deserving of fuller investigation.

Various dilutions of this dye were used according to the experiment, but a 1-100,000 strength was most generally employed. Latterly a 1-50,000 dilution has been adopted as the standardised strength.

Experiment 2.—To determine the ratio of inhibition, using a pure culture of *B. pullorum* on brilliant green and ordinary agar plates.

A standard emulsion, using a 48-hour growth of *B. pullorum*, was prepared to Tube 1 of McFarlane's nephelometer, ·02 cubic centimetre being the inoculum. Four plates were used for each, the inoculum being spread from Plate 1 to Plates 2, 3, and 4 without recharging. The resulting colonies were counted on a Pake's enumerating disc. Innumerable colonies were found on Plates 1 and 2.

Plates	*Brilliant Green* (1-100,000)	*Ordinary agar.*
1	Innumerable colonies	Innumerable colonies
2	Innumerable colonies	Innumerable colonies
3	416 colonies	976 colonies
4	176 colonies	400 colonies

This shows in 3 and 4 a ratio of 1 : 2·34 and 1 : 2·27 respectively. Plates 3 and 4 of the duplicate series confirmed these results, the actual ratios being 1 : 2·25 and 1 : 2·29.

The growth on brilliant green agar medium thus shows a more than 50 per cent. limitation as compared with that on ordinary nutrient agar medium.

Bacillus pullorum colonies on brilliant green medium are small and discrete ; they appear translucent after 24 hours' growth, and after 48 hours possess a greenish opacity when held to reflected light.

Experiment 3.—To determine the inhibition of non-pullorum colonies as compared with pullorum colonies from the fæces of chicks approximately seven days old affected with bacillary white diarrhœa.

The emulsion of the fæces was 1-16 of saline, ·02 c.c. being added as the inoculum to Plate 1 in both cases.

Plates	*Brilliand Green agar* (1-100,000)			*Ordinary agar*
	Pullorum Colonies	*Non-Pullorum*	*Pullorum*	*Non-Pullorum Colonies*
1	Innumerable	3	Crowded out	Innumerable
2	Innumerable	1	Crowded out	Innumerable
3	320	0	Crowded out	500
4	43	0	124	164

It will be noted that the inhibition of *B. pullorum* is slightly more than that in the previous experiment, the approximate ratio being 1 : 3.

Comparison of the non-pullorum colonies is interesting ; in Plate 3 there were no contaminating colonies on brilliant green, whereas on ordinary agar they were innumerable.

Of 33 cases plated and recorded, 18 were free from contaminators, the growth of *B. pullorum* being pure and vigorous. Nine showed a few colonies of coliform bacilli, ranging from 1-12 colonies per plate. Only six showed definite signs of contamination, but in each instance it was a comparatively simple matter to " fish off " *B. pullorum* colonies. A heavy contamination on a plate was usually found to be due to only one type of organism.

(As a matter of interest, several of the contaminating colonies that occurred regularly on the brilliant green medium, when plated with fæces, were isolated, and their biochemical reactions noted. Some 50 per cent. of these were found to ferment adonite and inosite. The colonies of these organisms appeared large, raised, and slimy and viscid in nature. These probably belong to the group of organisms referred to by Mackie and McCartney[3] as " Inosite fermenters.")

Experiment 4.—To determine the viability of *B. pullorum* in fæces.

Chick fæces collected from 24 outbreaks of bacillary white diarrhœa were emulsified in 1-4 of sterile normal saline which contained a few drops of glycerine and retained in test tubes labelled with the name and date of collection. Cultures from the fæcal emulsion from each of the 24 tubes were made on brilliant green plates every third day, and colonies were picked off and confirmed by the usual methods. Ordinary agar plates were inoculated similarly and used as controls in each case. This process was carried on until no further pullorum colonies were isolated from the plates.

In two cases colonies of *B. pullorum* were isolated from fæcal emulsions which had been retained for more than three months. The period of viability in the great majority of cases fell between 50 and 90 days ; in two cases only were periods of less than 50 days recorded, these being 30 and 32 days respectively. On the other hand, the maximum period recorded was 101 days.

The ratio of contamination did not vary during the experiment, and many of the plates developed a pure growth of *B. pullorum* until that organism had apparently died out in the fæcal emulsion. The ordinary agar plates were completely overgrown with coliform and Gram-positive organisms.

This raises the question as to how long *B. pullorum* can survive in a hen run or in the droppings of affected birds. Certainly 101 days appears to be a somewhat lengthy period for *B. pullorum* to live amongst the toxic products of the fæces, but the experiment seems to indicate that *B. pullorum* can remain alive for a very long time in moist fæces.

Experiment 5.—To determine how long after death of the chicks *B. pullorum* can be recovered from the putrefying carcases.

For this experiment 24 chicks which had died of bacillary white diarrhœa were retained at room temperature.

These chicks were post-mortemed, two at a time, at three-day intervals, and cultures were made on brilliant green and ordinary nutrient agar plates from the liver and fæces.

Pure growths of *B. pullorum* were obtained on the brilliant green plates up to and including 30 days after death. At this stage the chicks had become so putrid that the inoculum consisted of a platinum loopful of abdominal

contents. The experiment was then discontinued, as no immediately useful purposes were to be served by its continuation. The ordinary agar plates were completely overgrown with coliform and Gram-positive contaminators.

While the experiment does not show the extreme limit of time up to which *B. pullorum* can be recovered from putrefied chicks, it does show that by using a brilliant green medium it is possible to recover *B. pullorum* in pure culture up to and including 30 days after death, and when the chicks have become extremely decomposed.

It may also be recorded here that pure cultures of *B. pullorum* were obtained on brilliant green medium from the fæces of chicks in three different outbreaks of bacillary white diarrhœa when ordinary routine examination (*i.e.*, liver cultures) had failed to show the presence of the organism. Fortunately, these chicks had been retained, and duplicate cultures were prepared from the liver as a check, but the results were again negative, thus showing that the organism is not always recovered with equal facility from the liver and yolk sac. In such cases the existing routine methods will fail to show the presence of *B. pullorum* as the cause of death. This possibility and the circumstances of its disclosure seem to indicate that a fæcal examination might usefully be added to the existing laboratory practice in cases of suspected bacillary white diarrhœa. On the other hand, out of 116 outbreaks confirmed, fæcal examination failed to reveal nine, a percentage of failures of 7·8. In at least three of these cases there is strong reason to suspect a deterioration of the stock solution of the inhibitory agent as the cause of failure. A further reference to this aspect is made later.

The addition of brilliant green plating of fæces to the existing routine laboratory examination for the diagnosis of bacillary white diarrhœa should give much more reliable results and decrease the percentage of error.

The routine plating of fæces has lately been adopted in this laboratory, and incidentally has been applied in the examination of fæces from young turkeys ; it is interesting to note that a number of deaths in turkey chicks was found to be due to *B. pullorum* infection. The liver cultures and the fæcal plating showed typical growths which were confirmed by the usual methods.

A natural extension of the work outlined above was its possible application in the case of adult fowls and to members of the typhoid group of organisms.

The Cultural Examination of the Fæces of Adult Fowls on Brilliant Green Plates for Members of the Typhoid Group of Organisms.

Fourteen fowls were examined, seven of which were known reactors to the agglutination test for *B. pullorum*, and two were from a known fowl typhoid flock. No information was available concerning the other five birds.

A two-millimetre loopful of fæces from the posterior portion of the bowel was used for plating in the case of a dead specimen, and from the excreta in a live fowl. This loopful was added to the plate and mixed with a loopful of sterile saline. Only rarely did a heavy contaminating growth result from the plating of such fæces.

Examination of Adult Fowls.

No.	*Agglutination Test.*	*Ovaries.*	*Fæces.*
1	Pos. 1-100	Negative	Positive
2	Pos. 1-100	Negative	Negative
3	No record	Negative	Negative
4	Pos. 1-400	Positive	Postive
5	Pos. 1-25	Positive	Negative
6	No record	—	Negative
7	No record	—	Negative
8	Pos. 1-100	Negative	Negative
9	No record	Negative	Negative
10	No record	Negative	Negative
11	No record	—	Negative
12	Pos. 1-100	Positive	Positive
13	Nil. Fowl typhoid	—	Positive
14	Nil. Fowl typhoid		Positive

As will be observed, *B. pullorum* was recovered from the fæces of three of the specimens, and *B. sanguinarium* from two, the organisms being identified by cultural, biochemical, and serological tests.

In two of the three cases of *B. pullorum* the organism was also recovered from the ovaries, the same method of plating being adopted.

It will be noted that while No. 1 showed a good growth on the examination of fæces but failed to show the presence of the organism by ovarian examination, the reverse of this was found in No. 5, where fæcal examination failed to reveal the organism although its presence had been determined in the ovary.

No. 1 was a case of chronic diarrhœa, and the evidence shows that such chronic diarrhœa may be due to an intestinal infection with *B. pullorum.*

More than ordinary interest attaches to No. 12. It was a dead fowl received from an outbreak of bacillary white diarrhœa amongst adults, and showed characteristic lesions of pneumonia, myocarditis, and, most noteworthy, a blood-stained enteritis from which a heavy growth of *B. pullorum* was recovered on the brilliant green plates.

Nos. 13 and 14 were live fowls received from a flock in which fowl typhoid had been confirmed ; these were light and unthrifty, with pale combs, but were supposed to have recovered. The original purpose of the examination was the recovery of a bacteriophage from the droppings, but routine plating was done as a precautionary measure. On both sets of plates a heavy growth of *B. sanguinarium* was obtained, free from contamination, showing that the droppings contained immense numbers of the organism.

In each of the four cases in which *B. pullorum* was recovered the fowl had reacted to the agglutination test.

Fowl Typhoid.

B. sanguinarium grows readily on brilliant green, but the colony is larger than that of *B. pullorum*, and possesses a brownish tinge instead of the clear translucent appearance of the latter when held to

transmitted light. In three outbreaks of fowl typhoid plating of fæces was carried out, and the organism responsible was found in each instance, a good growth being obtained. In this connection it may be mentioned that an outbreak of deaths amongst chicks due to *B. sanguinarium* was encountered in the same flock as Nos. 13 and 14 (examination of adult fowls). The organism was isolated from the fæces and liver and identified by the usual methods.

Notes on the Use of Brilliant Green.

As recommended by the Medical Research Council[2], brilliant green in a fluid medium may be used with great success in human fæcal examination for members of the typhoid group. The fluid medium consists, in the Single Tube Method, of :—

Peptone water *p*H 7·2	..	..	100 c.c.
Brilliant Green, 1-1,000	..	..	0·5 c.c.

This medium was modified to suit the requirements of the investigation, and 2 c.c. of a 1-1,000 solution of brilliant green was added instead of a ·5 c.c., this making a dilution of 1-50,000, which was found to be more suitable for the examination of chick fæces.

Method.—A tube containing 10 c.c. of the fluid medium is inoculated with two or three loopfuls of fæces, and incubated for six hours at 37° C. A drop of this is subcultured on to a differential medium such as McConkey's, or brilliant green. In practically all cases this method yields a pure growth of *B. pullorum*.

There is a good deal of variation in the quality of brilliant green obtainable, and some types are unsuitable for use in this connection. It is essential that " brilliant green sulphate crystals, zinc free," should be specified (McLeod[1]).

Many control experiments were carried out, using different makers' products. Grüblers (Leipzig), Baird and Tatlock (London), and Nobel's (Imperial Chemicals) were amongst the samples that showed efficient inhibition.

Stock solutions of 1-100 or 1-1,000 can be made and used daily, but not more than one week's supply should be made up at a time. The period of reliability of a stock solution of brilliant green is often set at one month, but in the writer's experience this is too long and the most dependable results are obtained when the solution is not older than one week. The nature of the change which takes place in solution and reduces the inhibitive power of the dye is obscure.

Although solid media of *p*H 7·4 were used throughout the experiments, other observations seem to indicate that a *p*H variation between 7·00 and 7·6 has little influence on the inhibitive effect of the dye.

Plates after growth of the organism and several days' incubation tend to lose their green colour, due to the acid reaction of the medium, and at this stage fungi and Gram-negative organisms previously inhibited begin to grow.

There are several obvious minor advantages which the use of brilliant green offers, particularly where a large number of cases are being handled. The selective and strongly inhibitive properties render the excessive manipulative care required for ordinary agar plates unnecessary. For example, the lids can be safely left off the plates to hasten setting and drying of the medium.

The Use of Telluric Acid in conjunction with Brilliant Green.

Though not essential for satisfactory results, the use of telluric acid in conjunction with brilliant green is worth consideration in that the effect of telluric acid in colouring contaminating colonies acts as an additional safeguard and makes the presence of any such colonies more obvious.

Conclusions.

1. That the routine plating of fæces on brilliant green agar plates is a valuable aid to the diagnosis of bacillary white diarrhœa, and, when used in conjunction with existing laboratory routine methods, will reduce the percentage of error in diagnosis very considerably.
2. That brilliant green inhibits a great many of the coliform group which are normal saprophytes in the intestinal canal of even very young chicks, and that it inhibits all Gram-positive organisms.
3. That brilliant green media may be used successfully for the diagnosis of " carriers " among adult fowls. *B. pullorum* may be present in the intestinal canal, producing a chronic enteritis, and yet not be present in the ovaries.
4. That brilliant green fluid medium may be used with great success in conjunction with a differential medium for the examination of the fæces of chicks.
5. That *Bacillus pullorum* may remain viable in moist fæces for a period of 101 days.
6. That *Bacillus pullorum* may be found in the fæces when liver cultures have given negative results, thus explaining the occasional failure to diagnose bacillary white diarrhœa when the mortality is due to *B. pullorum*.
7. That the selective media may be used for the diagnosis of *B. pullorum* from carcases in all stages of putrefaction, up to and including 30 days after death.
8. That brilliant green medium may also be used in the diagnosis of fowl typhoid and in demonstration of " carriers " of this disease.

References.

[1] McLeod. Journal of Hygiene, 1919, Vol. 18, p. 260.
[2] Medical Research Council : Special Report, 51, 1920.
[3] Mackie and McCartney. " Introduction to Bacteriology," 1925, 201.
[4] Stafseth and Mallman. " Selective media," Poultry Science, 1928, Nov.

REVIEWS.

The Prevention of Human Tuberculosis of Bovine Origin. By William G. Savage, B.SC., M.D. (LOND.), D.P.H., County Medical Officer, Somerset. London : Macmillan and Co., 1929. Pages 195. Price 10s. 6d. net.

This work can be highly recommended to members of the medical and veterinary professions, and even to laymen, who desire to be informed regarding the facts of which knowledge is essential in considering the problem expressed in the title In the first four chapters the author deals concisely and lucidly with the vehicles of the infecting bacilli, the amount of human tuberculosis of bovine origin, the frequency of the disease in cattle, and how to reduce it and diminish the risk of infection through milk. The remainder of the work discusses technical problems relating to bacteriological and veterinary control, immunity in relation to bovine tuberculosis, and pasteurisation of milk. In the final chapter the author gives his own estimate of the relative value of the different methods of dealing with the problem that are now in force or have been recommended. He is of the opinion that any eradicative scheme based on tuberculin testing and compulsory slaughter of reacting animals is entirely inapplicable at present in Great Britain, which is the logical conclusion from the facts exposed in the body of the work. The existing Tuberculosis Order is adversely criticised, but perhaps without allowing adequate weight to the fact that it has never been claimed that the Order would have any serious effect on the incidence of the disease in cattle, but only that it eliminates from human consumption a considerable amount of milk containing bovine tubercle bacilli. With regard to systematic veterinary inspection of dairy cattle, the author suggests that " it must stand or fall on its merits as a weapon for the elimination of bovine tuberculosis." But here, again, the matter is not fairly stated. Systematic veterinary inspection of dairy cattle, no matter how frequent, could never by itself eradicate bovine tuberculosis and it must stand or fall not by its success or failure in that direction but by its effect in the detection of the cows visibly affected with tuberculous mastitis or otherwise clinically recognisable as tuberculous, which animals are responsible for the majority of cases of human infection with bacilli of the bovine type.

Dr. Savage thinks that pasteurisation of milk to prevent the infection of human beings with tubercle bacilli of bovine origin is a confession of failure, and he considers routine veterinary inspection of dairy cows not worth doing, but, apparently, that is because he has no patience with any procedure that does not clearly contribute to the eradication of bovine tuberculosis, and, as previously mentioned, he regards the only method that experience and reason show to be widely effective in that direction as impracticable in this country. A reader will naturally pass on from those passages in lively expectation of finding that the author himself has some entirely new scheme certain or likely to cause the disappearance of tuberculosis as a disease of cattle and not open to the defects with which he reproaches existing methods of dealing with the problem. In that, the reader is likely to experience disappointment, since, for the most part, the procedures suggested are only in the direction of strengthening those already in force, such as improvement in the hygienic conditions under which cows are kept, educational efforts to enlist fuller co-operation on the part of the farmer, and increased penalties for neglecting to notify the suspected existence of the disease. Apparently, even the demolition of cowsheds is contemplated,

or their retention only for milking, or for housing under exceptionally severe weather conditions. There is also thought to be a " gleam of hope " in immunisation of bovine animals against the disease. Finally, however, the author is driven to the conclusion that failing improvements in the directions he suggests, it would " be better for those concerned with the human infection side to face their own problem in their own way, to leave bovine tuberculosis to those concerned with animal diseases, and to protect the milk supply by an efficient and adequately controlled system of pasteurisationion." But, obviously, the first category at present includes all those in this country who use milk as part of their diet, and it would not have been out of place to remind them that a household practice of steaming the milk would be more certain than pasteurisation and probably cheaper.

Diet and the Teeth : An Experimental Study. Part I. Dental Structure in Dogs. By May Mellanby. Special Report Series, No. 140, Medical Research Council. H.M. Stationery Office, 1929. Price 17s. 6d. net.

This publication records the results of a prolonged and painstaking research concerning the normal dentition of the dog, and the departures from the normal in teeth development and structure which occurred under certain experimental conditions, generally involving a diet in some respect defective. The work was not undertaken with special regard to abnormal dentition or structural disease of the teeth of the dog, but in the hope that it might throw light on similar abnormalities in the human subject, particularly caries, which hardly lends itself to experimental investigation. It is admitted that results obtained in experiments with monkeys might have been more directly applicable to man, but it would not have been possible to use these animals on a large scale, and, everything considered, puppies were selected as the best for the purpose. For the most part, these were bred for the purpose, a method that had among other advantages that of employing strictly comparable animals of the same litter for experiment and for controls.

The experiments and observations have established a definite relationship between defectively developed or calcified teeth in dogs and rickets. The dietetic substances which are of importance in this connection are generally those that have a high content of vitamin A, but that is recognised to be distinct from the factor specially concerned in calcification and now known as vitamin D, or the anti-rachitic vitamin. The development of perfect teeth can be ensured in puppies by an adequate supply of the latter either in natural foods, such as egg-yolk, milk, and suet, or in cod-liver oil ; or as artificially produced in foods or even by irradiation of the animal's skin. In the basal diet of the puppies the main ingredient was a cooked cereal amounting to from 50 to 75 per cent. of the whole. An important fact discovered was that cereals contain a chemical entity of unknown composition which exerts an anti-calcifying effect, and the experiments indicated that among the cereals used this injurious agent was most abundant in oatmeal and least abundant in white flour.

The experiments bearing on the point indicated that exercise plays little or no part in the development of the teeth of the dog, but it is said that exposure to sunlight may result in the formation of good teeth with diets which would otherwise produce somewhat defectively calcified teeth.

The experimental results described in the report are said to show that it is now possible to produce at will any degree of dental structure from the most perfect to the most imperfect, mainly by regulating the diet. Fortunately it may still be said that it appears to be difficult to ensure bad

calcification of teeth or bones in puppies with an adequate amount of an ordinary diet, provided they have a reasonable amount of exercise with exposure to sunlight.

Special mention ought to be made of the 109 photographs and photomicrographs which admirably illustrate the development and structure of the teeth under normal and abnormal conditions.

The Internal Secretions of the Ovary. By A. S. Parkes, M.A. (CANTAB.) PH.D. (MANCH.), B.SC. (LONDON). London : Longmans, Green and Co., 1929. Pages 242. Price 21s. net.

This work forms one of the series of monographs on Physiology by the same publishers and maintains the high standard of the others. Whether the ovary forms an internal secretion or hormone that is responsible for the cyclical changes in the uterus and vagina and the development of the mammary glands in pregnancy, and whether this substance can be extracted in a fully active condition from the ovaries and usefully employed in medical or veterinary practice, are the questions with which it deals. At the outset, there is a very condensed description of the normal ovary and the accessory organs of reproduction in the female, followed by chapters dealing with sexual periodicity and the different types of the œstrus cycle in different species of animals. The remainder of the work sets forth the evidence regarding the existence of one or more ovarian hormones, including the various methods by which extraction has been attempted and the observed effects of the extracted substances in experiments on animals. How difficult it is to interpret the experimental results is well shown, but the author ventures to think with some confidence that there are at least two separate ovarian hormones. The first of these is the œstrus-producing hormone, or œstrin, which, it is claimed, is capable, when injected in adequate doses, of producing all the extra-ovarian histological and physiological symptoms of the normal œstrus period, except possibly the mating instinct. It will be noted that the exception mentioned would be a very important one in veterinary practice. The second hormone is œstrus-inhibiting in effect, and is furnished by the corpus luteum, though it may occur in small amount in the blood of pregnant animals. It also appears to sensitise the uterus for the implantation of the fertilised ova, to maintain pregnancy, and to promote development of the mammary glands. A very important fact, only recently discovered, is that precocious œstrus can be produced in the ovaries and accessory organs of normal immature animals by administration of anterior pituitary substance, which suggests that this organ plays some part in regulating the normal ovarian cycle and may be the determining cause of the first œstrus of puberty.

There are 68 excellent illustrations in the text and a very complete bibliography. The publishers' share of the work has been well done.

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